VOLUME LXIV NUMBER 3

THE
BOTANICAL GAZETTE

SEPTEMBER 1917

ROOT SYSTEMS OF CERTAIN DESERT PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 236

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(WITH THIRTY-THREE FIGURES)

Introduction

Although the aerial parts of plants have long been studied, little was known of the nature of the subterranean parts until the work of CANNON[1] upon the plants of the region about Tucson, Arizona. The supposition had been that in general the roots of desert plants are of great length and deep penetration. The work of CANNON showed that while this is sometimes true, such roots are often of no unusual length and in some cases are very superficial.

Since the soil conditions and the flora of the vicinity of Albuquerque are different from those at Tucson, a study of the roots of plants was undertaken in that vicinity, during the first half of the year 1915. Albuquerque lies in the valley of the Rio Grande, on each side of which are bluffs, leading to an apparently level upland. The mesa on the east rises from an elevation of 200–300 ft. above the valley floor to an elevation of 1200–1500 ft. at the base of the Sandia Mountains 10 miles farther east. This mesa is composed to a great depth entirely of unconsolidated materials, as is shown by a boring of the Water Supply Company 725 ft. deep.[2] It is

[1] CANNON, W. A., The root systems of desert plants. Publ. Carnegie Inst. Wash. no. 131.

[2] BRYAN, K., Geology of the vicinity of Albuquerque. Bull. Univ. N. Mex. no. 5.

concluded from this that previous to the deposition of these beds there was here a deep trough, which other evidence shows to have been the bed of a large river, which existed at a period of greater precipitation and was subsequently filled with stream-borne material. On account of its fluviatile origin the material is extremely variable, being composed of layers of sand, adobe, clay, gravel, boulders, and combinations of these materials, with marked local variations, both horizontally and vertically. On this mesa and the numerous arroyos which dissect its edge grew the plants studied.

One of the principal features of the soil in the habitats described by CANNON is a thick layer of hardpan, or *caliche*, beginning at a depth of about 30 cm. and extending indefinitely. This is so hard as to prevent root penetration, except through cracks. Such layers are common in arid regions, and are formed, according to the opinion of CANNON and others, through the concentration of salts left by the evaporation of ground water gradually ascending by capillarity. Rainfall dissolves these materials, carrying them downward. These two processes result in the formation of a gradually increasing zone of precipitation. In the Albuquerque region this zone is very poorly developed, often being noticeable in a fresh exposure only by the presence of a whitish streak or pebbles stained with lime. When dry, such soil becomes very hard, and it is evident from the appearance of roots entering it that it offers considerable resistance to root penetration, but does not prevent it. When wet the hardpan is soft and easily penetrable. Under natural conditions, however, it is generally very dry.

The Albuquerque region is even more arid than that at Tucson, an average for 10 years giving a precipitation of 7.44 in., as compared with an average of 11.17 for Tucson. Much of this small amount is lost by run-off, since most of the precipitation occurs as torrential summer rains. Table I shows the localization of the rains in the summer months. The marked deviations from the average that are characteristic of individual years are shown by the precipitation during the time of this study in 1915. The lower temperature and the smaller amount of precipitation in the winter

months prevent the growth of the winter annuals so characteristic of the Tucson region.

TABLE I

RAINFALL

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Tucson	0.79	0.90	0.77	0.27	0.14	0.26	2.46	2.66	1.16	0.64	0.81	1.00	11.17
Albuquerque, average	0.48	0.33	0.22	0.26	0.69	0.35	1.43	1.07	1.70	0.77	0.46	31	7.44
Albuquerque, 1915	0.68	0.56	0.51	2.05	0.00	0.00	....	....	....	....	....	....	.....

TEMPERATURE

	1893	1894	1895	1896	1897	1898	1899	1900	1901	1902
Absolute minimum	11	0	10	13	2	0	1	9	4	12
Absolute maximum	98	95	95	100	95	104	104	101	99	100

EVAPORATION FROM A FREE WATER SURFACE

Jan.	Feb.	March	April	May	June	July	August	Sept.	October	Nov.	Dec.
2.04	2.63	6.17	6.82	10.08	12.63	11.78	10.21	8.00	4.38	1.73	1.40

The data for Tucson are given by COVILLE and MACDOUGAL[3] and those for Albuquerque by MAGNUSSON.[4]

A series of determinations of soil moisture was begun, but it was soon found that such data are of little value, since on account of the fact that the soil is composed of stream-borne material, it is subject to extreme local variations, even within the habitat of a single plant, as is shown in the descriptions of the habitats of most of the plants given in this paper. Samples of soil from different levels in the habitat of *Atriplex canescens* showed the following variations in moisture content, the percentages being based upon the dry weight of the soil. Coarse gravel at a depth of 1 ft., 4 per cent; fine sand at 2.5 ft., 10 per cent; hardpan at 4 ft., 3 per cent. Since the precipitation during the time of this study was

[3] COVILLE, F. V., and MacDougal, D. T., Desert botanical laboratory of the Carnegie Institution. Publ. Carnegie Inst. Wash. no. 6.

[4] MAGNUSSON, C. E., Bull. Univ. N. Mex. no. 5.

several times the normal amount, the figures would have had even less value than usual. Similar variations in water holding capacity and wilting coefficient would result from the lack of uniformity in the soil.

On account of climatic differences, the flora is very different from that of the Tucson region. The larger cacti are absent here, all being low forms, excepting *Opuntia arborescens*. No tree is found on the mesa or its arroyos, and the bushes, except *Chilopsis saligna*, are seldom more than 5 ft. in height. The period of greatest growth follows the rainy season, whenever it may occur. There are no winter annuals here, the corresponding forms being either biennials or summer annuals. The annual plants are greatly in the minority, the most conspicuous part of the flora being composed of perennial herbs.

The principal plant associations occurring within the area studied are as follows.

1. The *Bouteloua* association, characterized by the grama and other grasses and formerly occupying the entire mesa, but now much invaded by ruderals, such as *Gutierrezia Sarothrae* and *Salsola*.

2. The *Dysodia-Ephedra* association, dominated by *Ephedra trifurca* and *Dysodia acerosum*. The majority of the plants have reduced aerial parts and a large root system. The association occupies the top and the upper portion of the sides of the gravelly ridges between the arroyos, where exposure and run-off are maximum. The soil is often pure gravel and the surface is generally a mosaic of pebbles.

3. The *Chrysothamnus* association, dominated by *Chrysothamnus Bigelovii* and occupying the lower parts of the sides of the arroyos. The soil is generally adobe, often with much sand and gravel.

4. The *Fallugia* association, dominated by *Fallugia paradoxa* and forming a narrow fringe along the beds of arroyos, which are generally covered with loose sand and gravel.

Method of work

Annuals, biennials, and some of the smaller perennials were removed from the ground and photographed. On account of the difficulty of obtaining the delicate ultimate parts of the roots,

many are necessarily incomplete. It was found possible to photograph some of the larger perennials *in situ*, but in general it was found much more satisfactory to make accurate diagrams of the horizontal and vertical extensions of the root systems on paper metrically ruled in squares. The diagrams appear here drawn to a scale of 1:20, except figs. 25 and 26. While in general only the roots lying in or near a particular plane are represented, it was generally found possible without much distortion to include the most important part of each root system in both the horizontal and vertical diagrams. Roots that for some reason were not followed to the end are terminated in the diagrams by a broken line. Roots turning to a direction at right angles to the plane of the diagram end in a dot.

CANNON divides root systems into 3 types: (1) a generalized type, in which there is a well balanced development of both tap and lateral roots; (2) a specialized form, in which the tap root is much the more prominent; and (3) a second specialized form, characterized by a relatively better development of the lateral roots. This classification is used in the present paper.

Description of root sytems

ANNUALS AND BIENNIALS

Biscutella Wislizeni.—This plant is common in sandy situations on the mesa and in the arroyos. The root system consists of a relatively short tap root, with many strongly developed laterals extending horizontally rather near the surface of the soil. Some of them usually exceed the tap root in length. The specimen shown in fig. 1 grew in a sandy arroyo bed and was about 10 in. in height.

Phacelia corrugata.—This plant is common in a number of associations. It generally occurs as an annual, although specimens in favorable situations may start in the fall and form rosettes which survive the winter. The leaf structure shows almost no xerophytic characteristics, there being only one layer of palisades, large air spaces, and a thin epidermal wall. Considerable variation of the root system occurs under different soil conditions. The plant

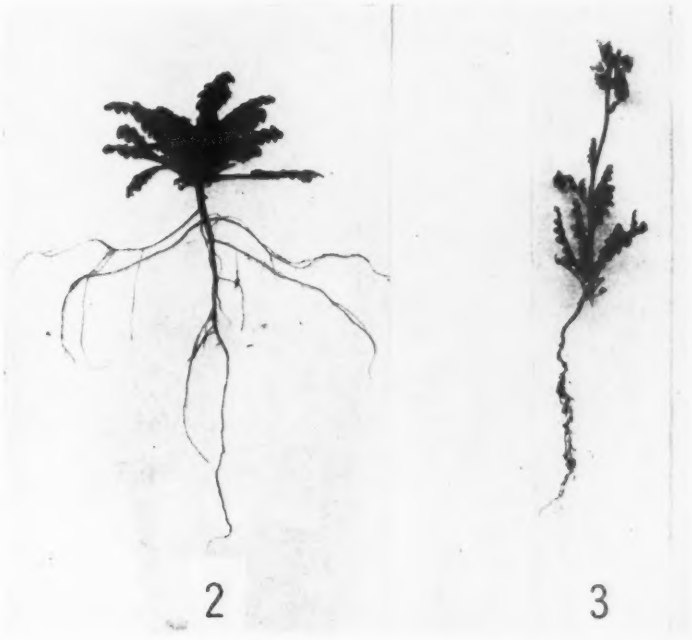
shown in fig. 2 grew in a moist situation, where the water of an arroyo was impounded by a dam. The plant is in its early spring condition, the rosette having survived the winter. The root system is of the generalized type. The laterals near the surface are especially well developed, sometimes exceeding the tap root in length. The specimen shown in fig. 3 is from a dry, gravelly ridge in the *Dysodia-Ephedra* association. The impoverished condition of the plant is shown by the weak development of both root and shoot. Here the tap root is relatively the more important.

FIG. 1.—*Biscutella Wislizeni*

Allocarya crassisepala.—This is one of the most common annuals of the mesa and arroyos and is a ruderal in a number of associations. The plants vary very greatly with the soil conditions in both root and shoot. The plant shown in fig. 4 grew in moist soil in the bottom of an arroyo and bore numerous prostrate branches. The root system was superficial and consisted of a short tap root which soon became horizontal, and a number of long, branched laterals arising about an inch below the surface of the soil. The plant shown in fig. 5 grew in dry soil and had a tap root relatively well developed and vertical, with smaller and less numerous laterals.

Sisymbrium canescens.—This plant is common in a number of associations. It makes a quick growth and maturity after a period of rain. The root system is very small in proportion to the shoot and consists of a tap root with a number of small laterals.

Linum rigidum.—This plant is characteristic of the *Dysodia-Ephedra* association. Both root and shoot are much reduced. The root system in the plant shown in fig. 7 consisted of a tap root about 5 in. long and a few short laterals with almost no fine ultimate branches.



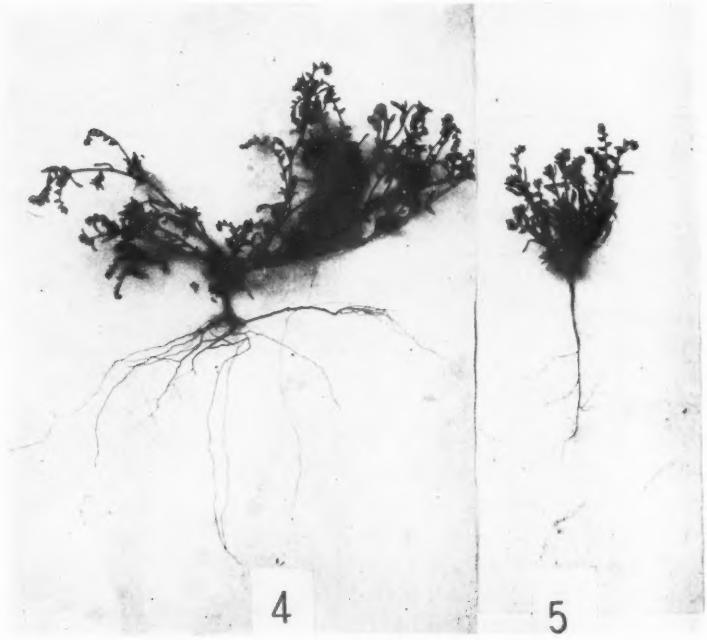
FIGS. 2, 3.—Fig. 2, *Phacelia corrugata* from moist habitat; fig. 3, *P. corrugata* from dry habitat.

PERENNIAL HERBS

Cymopterus Fendleri.—This plant is exceedingly common on the mesa and the ridges between the arroyos. It is the earliest plant to begin growth and to blossom in the spring. The leaves are in the form of a loose rosette and are xerophytic in structure. The root system is characterized by a thick, fleshy tap root which occasionally bears one or two fleshy laterals. In the growing season there appear numerous slender, white, absorptive roots

which later are deciduous. These always break off upon removal from the soil and do not appear in the figure.

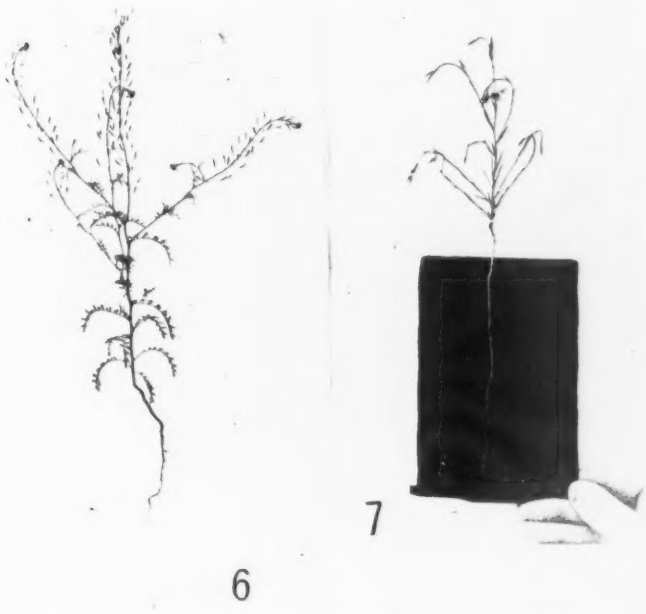
Rumex hymenosepalus.—This plant is common in the more mesophytic places on the mesa, especially in broad, shallow arroyos. The rosette of broad, thick leaves appears very early in the spring and is soon followed by a spike of flowers. The root is very large



FIGS. 4, 5.—Fig. 4, *Allocarya crassisepala* from moist habitat; fig. 5, *A. crassisepala* from dry habitat.

and fleshy. Young roots are constantly being formed as the oldest decay. The irregular projections at the top of the root shown in fig. 9 represent 10 or 11 crowns of former years. The youngest roots bear short laterals, but the older ones are generally without them. It is interesting to note that of the plants studied the three having the most prominent storage organs in the way of thickened roots all grow in situations more mesophytic than the average.

Rumex is common in the arroyos on the mesa; *Cucurbita foetidissima* occurs most abundantly along the banks of irrigation ditches in the valley; *Berlandiera lyrata* is confined almost entirely to the sides of arroyo beds. Thus it appears that the plants having this "adaptation" to an arid environment are in less need of it than most of the plants of the region.



FIGS. 6, 7.—Fig. 6, *Sisymbrium canescens;* fig. 7, *Linum rigidum*

Astragalus diphysus and *A. mollissimus.*—These are common evergreen plants of the mesa. The root systems are similar and are characterized by prominent tap roots with a few large and several small laterals, which are generally deeply placed. The root tubercles are small and not numerous. A plant of *A. mollissimus* is shown in fig. 10.

Solanum eleagnifolium.—This plant is very characteristic of sandy situations and the rapidly eroding sides of arroyos. The

plant shown in fig. 11 grew in the latter situation. The upper 12 in. of soil was sandy adobe, followed by 10 in. of clayey adobe with some gravel. Below this was a layer of loose gravel about 2 in. in thickness, under which was fine sand to an unknown depth. The plant was 18 in. in height. The root system was of the specialized type with strongly developed tap root. There were a few well developed laterals, two of which arose at the junction of the layers of sandy and clayey adobe. Upon reaching the layer of loose gravel, one of these turned abruptly and proceeded horizontally in the gravel layer, possibly on account of the greater ease of penetration. Another took a rather tortuous course downward and was lost at a depth of 65 in., below the level of the bottom of the arroyo. This root had a length of 72 in., and since its diameter at the broken end was 3 mm., its total length was undoubtedly much greater. Numerous small laterals arose in the upper 12 in. of soil.

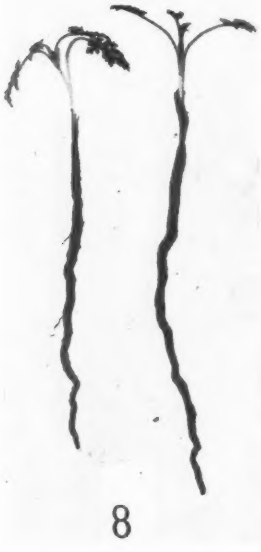


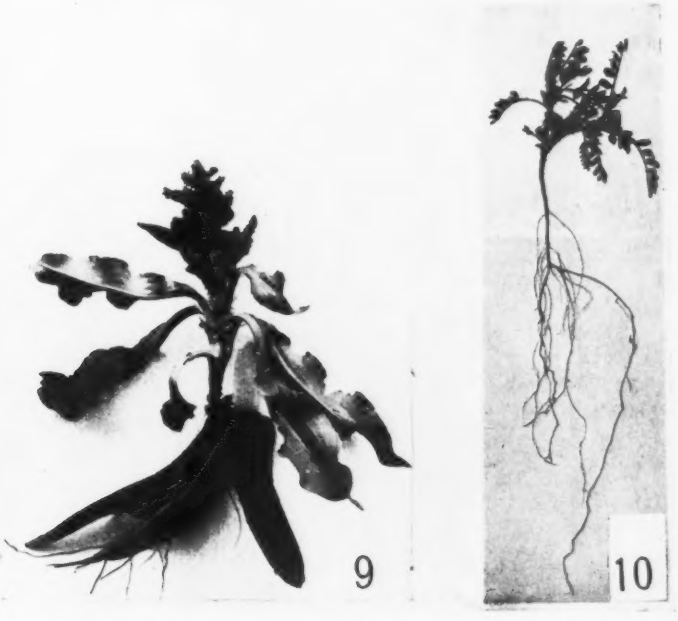
FIG. 8.—*Cymopterus Fendleri*

Cucurbita foetidissima.—This perennial herbaceous vine is common along irrigation ditches in the valley and less so along the sides of arroyo beds. The root is extremely thick and fleshy and is surmounted by an underground stem 6 in. in length. The main root of the plant studied was about 3.5 in. in diameter at the top and extended to a depth of 20 in., gradually becoming horizontal and forking several times after reaching a length of 36 in. The main root bore only one small lateral (fig. 12).

Pachylophus hirsutus.—This plant is a rosette in habit and is found in the driest and most exposed slopes of the gravelly ridges of the *Dysodia-Ephedra* association. The leaves are hairy and rather thick and succulent. The root system is fleshy and of

irregular form, without evident differentiation into tap root and laterals. The main root often proceeds horizontally and is little larger than the minor roots. The specimen shown in fig. 13 grew in pure gravel.

Euphorbia sp.—This small, prostrate plant grows on the sides of arroyo beds. The root system has much the same general form



FIGS. 9, 10.—Fig. 9, *Rumex hymenosepalus;* fig. 10, *Astragalus mollissimus*

as that of *Sphaeralcea*, but all the roots are slender, brown, and fibrous. The identity of the tap root is lost a short distance below the surface. The slender ultimate branches are very numerous. The root system is not deep, but very thoroughly permeates a limited amount of soil (fig. 14).

Sphaeralcea cuspidata.—This plant is very common in the *Boutcloua* and *Dysodia-Ephedra* associations. The plant shown in

fig. 15 grew in sandy soil on the mesa. The root system is characterized by a tap root which is poorly differentiated or even absent, and very prominent laterals. The remains of the crowns of several years are shown. When the plant grows in an unstable situation, such as the rapidly eroding side of an arroyo, vegetative reproduction from the roots occurs very commonly, enabling the plant to maintain a foothold.

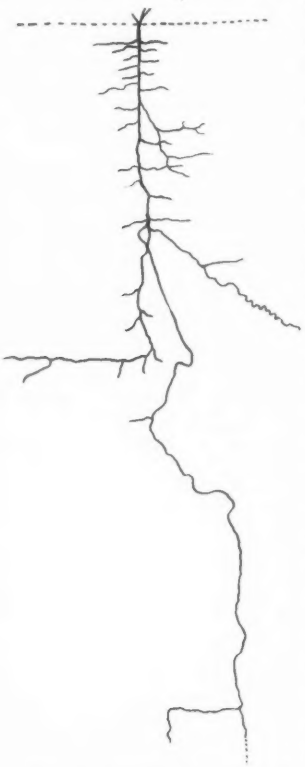
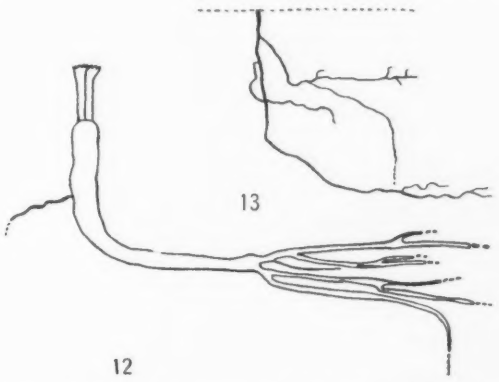


FIG. 11.—Vertical extension of root system of *Solanum eleagnifolium*.

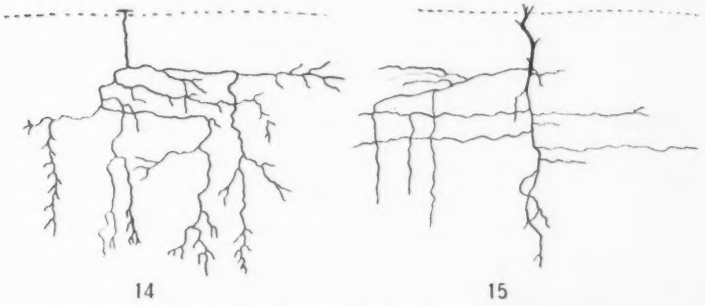
Berlandiera lyrata.—This plant is confined almost entirely to the sides of arroyo beds. The root system has a stout tap root with very few laterals. The group of laterals near the surface of the ground in so many plants of the region is absent here. The entire root system is thick and fleshy and the upper portion is swollen to the thickness of an inch. Plant *A*, fig. 16, grew about 1 ft. above the bed of an arroyo. The tap root divided into two horizontal branches slightly below the level of the arroyo bed. Plant *B* grew about 4 ft. above the bed of an arroyo and bore no extensive laterals until it reached the level of the bed of the arroyo, where it branched freely. The length of the tap root is apparently determined by the distance to the layer of moister soil on a level with the arroyo bed.

Gaura coccinea.—This perennial herb is very common on eroding arroyo sides and other disturbed situations. The root system belongs to the specialized type with prominent tap root. The length of the tap root is apparently determined by the distance of the plant above the arroyo bed. There are few laterals, most of

the absorption probably being done by the ultimate branches of the tap root. Vegetative reproduction from the roots is common (fig. 17).



FIGS. 12, 13.—Fig. 12, vertical extension of root system of *Cucurbita foetidissima;* fig. 13, vertical extension of root system of *Pachylophus hirsutus.*

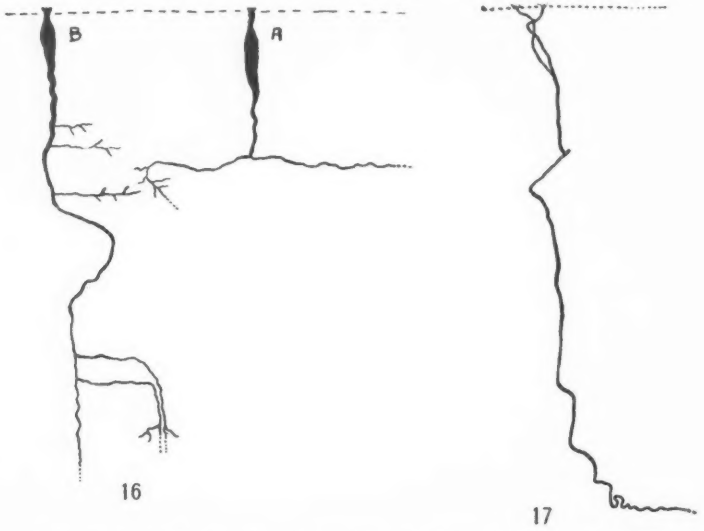


FIGS. 14, 15.—Fig. 14, vertical extension of root system of *Euphorbia* sp.; fig. 15, vertical extension of root system of *Sphaeralcea cuspidata.*

SHRUBS

Parosela formosa.—This low, much branched shrub is confined almost entirely to the sides of arroyo beds in the *Fallugia* association. The leaves are very small and xerophytic in structure. Several root systems were dug up and found to belong to the specialized type with prominent laterals, resembling somewhat the

cactus type. In some specimens no tap root could be distinguished, although a better development of the tap root was found in plants



FIGS. 16, 17.—Fig. 16, vertical extension of plants of *Berlandiera lyrata: A*, growing 1 ft. above arroyo bed; *B*, growing 4 ft. above arroyo bed; fig. 17, vertical extension of root system of *Gaura coccinea*.

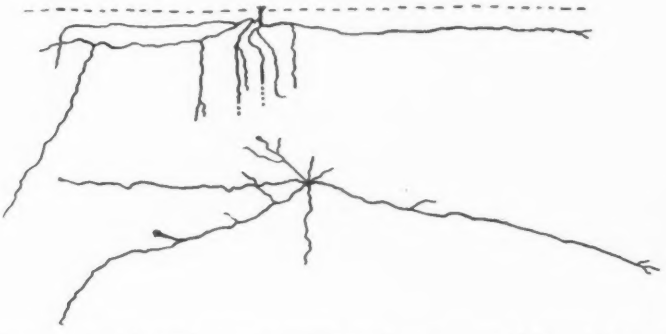


FIG. 18.—Vertical and horizontal extensions of root systems of *Parosela formosa*

growing higher above the arroyo bed. The plant shown in fig. 18 grew on the edge of a gravelly arroyo bed 6 ft. wide. The specimen

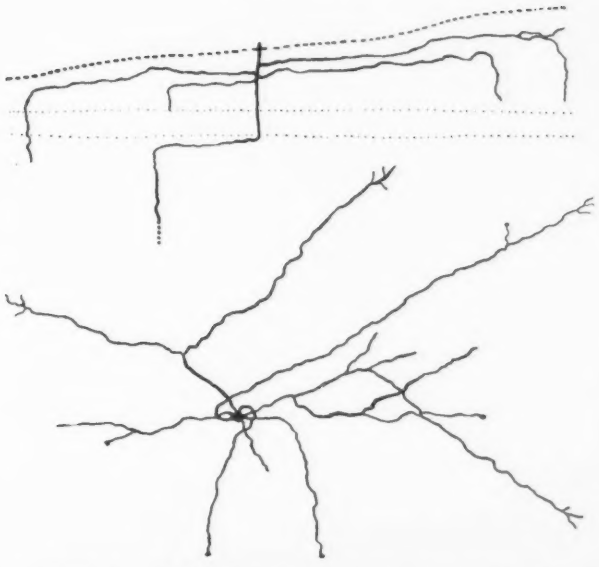


FIG. 19.—Vertical and horizontal extensions of root system of *Artemisia tridentata*

FIG. 20.—Root system of *Ephedra trifurca*

was 28 in. in height. The tap root was weak, being exceeded in diameter by several of the laterals. The laterals were numerous and arose just below the ground and proceeded horizontally 2 or 3 in. below the surface, most of them beneath the bed of the arroyo. The ends of several of them dipped abruptly downward. This type of root is rare in the region. The root system of *Parosela scoparia*, which grows in sand, was found to be similar, but more deeply placed.

FIG. 21.—Root system of *Dysodia acerosum*

Artemisia tridentata.—This plant grows along the sides of arroyos in the *Chrysothamnus* association. Well developed specimens are rare, since the plant is freely eaten by grazing animals. The specimen shown in fig. 19 grew in soil the upper 10 in. of which was adobe, overlying 12 in. of coarse gravel and pebbles, with adobe below this to an unknown depth. The tap root was well developed, but the laterals near the surface were extremely prominent. These were of two types: numerous short ones in the upper 12 in. of soil, and a few very long ones which arose from the upper 6 in. of the tap root. The latter proceeded horizontally 3–4 in. below the surface of the soil and reached a length of 20–40 in. There was a tendency for the ends of these roots to turn downward, as in *Parosela*.

Ephedra trifurca.—This is one of the dominant species of the *Dysodia-Ephedra* association. Investigation of a number of

specimens showed a considerable variation in the root system, which in general has a good development of both tap and lateral roots. The plant shown in fig. 20 grew in adobe soil about 2 ft. above the bottom of an arroyo and had prominent laterals and a stout but rapidly tapering tap root. Below the part shown in the photograph, two large laterals were given off, below which the tap root was insignificant. Another specimen growing in adobe soil 10 ft. above the bottom of an arroyo and exposed by erosion showed a relatively much greater development of the tap root. Several laterals, the largest half an inch in diameter, were given off in the upper 2 ft. of soil. Three small laterals occurred 3 ft. below the surface. The tap root proceeded somewhat tortuously downward to a depth of at least 11 ft., a little below the level of the arroyo bed, where a large lateral arose. Below this the tap root had a diameter of 5 mm. and was not followed farther. A third specimen grew on a hill and was exposed by the removal of gravel. The plant grew in a soil composed of boulders up to 8 in. in diameter, the interstices of which were filled with sand. The root system was essentially similar to the second specimen described. The cause of the variation in the length of the tap root apparently is not the character of the soil,

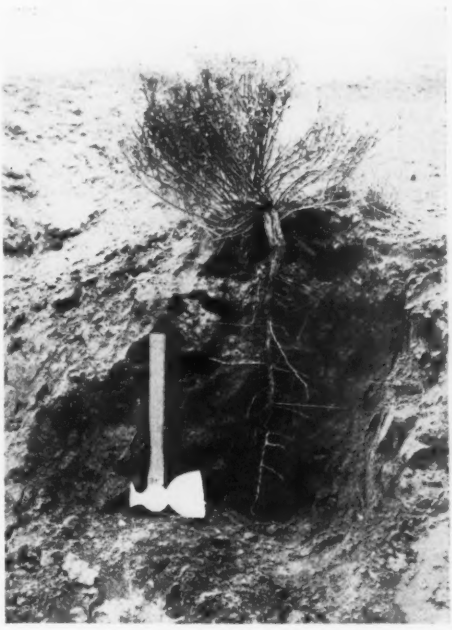


FIG. 22.—Root system of *Chrysothamnus Bigelovii*

but the height of the plant above the nearest arroyo. Even though there is a stream in the arroyo only a few hours each year, there is probably a layer of moister soil on a level with the bottom of the arroyo, on account of a slow creep of ground water toward the arroyo and the conservation of the moisture by the dry sand covering it.

Dysodia acerosum.—The habitat of this plant is the same as that of *Ephedra*. The aerial parts of the plant form a compact, much-branched tuft. The leaves are needle-like. The individual photographed grew in sandy adobe with large pebbles (fig. 21). The plant had a stout tap root with a few large laterals arising close together a short distance below the surface of the soil. The tap root shown measures 39 in., but it was probably several inches longer. Both tap and lateral roots bore numerous fine branches. This species probably has the largest root system in proportion to the size of the aerial parts of any of the plants of the region.

FIG. 23.—Root system of *Opuntia fragilis*

Chrysothamnus Bigelovii.—This is the dominant plant in an association characteristic of the lower parts of the sides of arroyos. The principal photosynthetic work is done by the almost leafless green stems. The root system is of the generalized type. Fig. 22 shows only a part of the root system of a rather young plant. Later excavation showed laterals up to 90 and 100 in. in length and a tap root about 100 in. long. The laterals are usually of small diameter

and taper very slightly and bear numerous small lateral branches, especially near their distal ends. Older individuals probably have root systems more extensive than that of any other plant of the arroyos or mesa.

Opuntia fragilis.—This is the smallest and most common cactus of the mesa, where large colonies form mounds of sand or adobe. The root system is very superficial and consists of one or two main roots with numerous small lateral branches (fig. 23).

Opuntia arborescens.—This is the only large cactus found in the region. It is characteristic of the pine-cedar association of the mountains and occurs sparingly on the mesa. The specimen shown in figs. 24 and 25 grew in the latter situation and was only 2.5 ft. in height. The root system is similar to the type described by CANNON for the larger forms occurring near Tucson. There is a sharp differentiation of absorptive and anchorage roots. The former are long and thin and occur within an inch or two of the surface; the latter are short and thick and deeply placed. The longest absorptive root had a length of 93 in.; the longest anchorage root a length of 30 in. The absorptive roots have many fine branches, especially near the ends.

FIG. 24.—Anchorage roots of *Opuntia arborescens*, vertical extension.

Opuntia camanchica.—This is the common prickly pear of the mesa and arroyos. The specimen shown in fig. 26 occurred in the *Dysodia-Ephedra* association in gravelly sand with small boulders. The plant had been formed vegetatively from a fallen segment which had become buried. The roots had originated from the pulvini and the proximal 2 inches of each was tuberous. The root system conforms to the usual superficial type described by CANNON for the smaller cacti, except for the presence of one thick, deeply placed root. The plant is usually several joints in height, so that

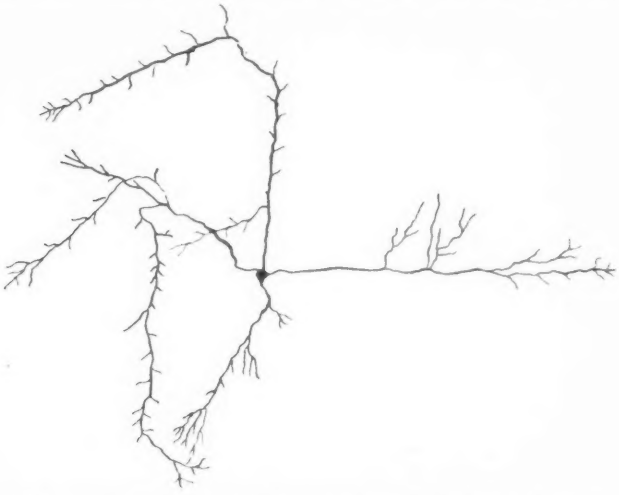


FIG. 25.—Absorptive roots of *Opuntia arborescens*, horizontal extension

especially in a strong wind there is considerable strain on the lower joint. The presence of the thicker, more deeply placed root may show a tendency toward the sharp differentiation of anchorage and absorptive roots so well shown by *Opuntia arborescens*.

Mamillaria Grahami.—This little cactus occurs sparingly in the driest parts of the *Dysodia-Ephedra* association. The root system differs from the usual cactus type in having a short but well marked tap root which bears many short and a few longer laterals.

Yucca glauca.—This plant is very common along arroyos on the mesa and in sandy soil. The plants generally grow in clumps

formed by vegetative multiplication. The root system consists of a thick, branched, horizontal portion bearing numerous laterals quite uniformly about 3 mm. in diameter. Those measured showed lengths of 5, 6, 7, and 24 in. The main root is usually about 2 in. in diameter and very succulent. It evidently functions as a storage organ.

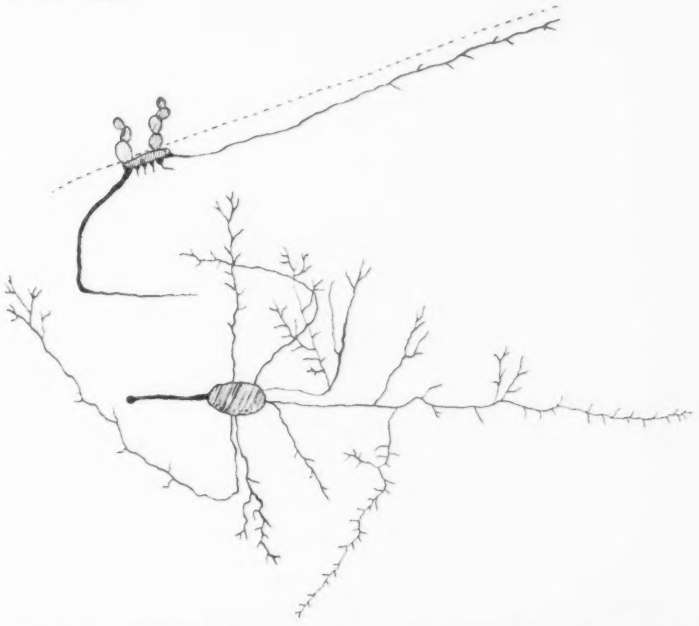


FIG. 26.—Vertical and horizontal extensions of root system of *Opuntia camanchica*

Eriogonum effusum.—This low shrub is characteristic of the edges of arroyo beds. The root system is of the generalized type. The plant shown in fig. 28 grew about 4 ft. above the bottom of an arroyo. The composition of the soil was as follows: upper 32 in., coarse sand and gravel; 10 in. of moist sand; dry gravel to an unknown depth. Several prominent laterals penetrated the sand and gravel layer. Two of the largest laterals occurred in the layer of moist sand. Another specimen, which grew about 10 ft. above the bed of an arroyo, had a tap root 80 in. long with the laterals

confined almost entirely to the space between the 16 and 24 in. depths. The effect of the position of the plant with reference to the arroyo bed is shown here.

Gutierrezia Sarothrae.—This semi-evergreen shrub is a common ruderal in many associations of mesa, mountain, and valley, but especially on the mesa, where grazing has been a greater disturbing factor. The plant shown in the photograph grew near the bottom of an arroyo and had a root system of the generalized type. The

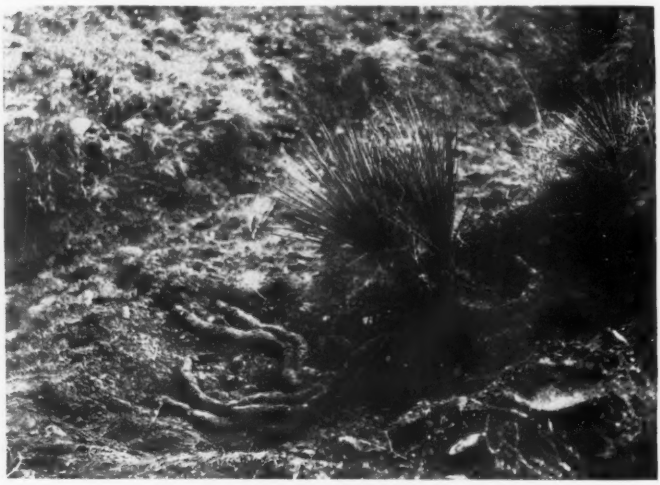


FIG. 27.—*Yucca glauca*

plant shown in the diagrams was a small specimen 8 in. in height and grew near the edge of the steep bank of an arroyo bed. The tap root was especially well developed and extended vertically to a depth of 44 in., where it reached the level of the bottom of the arroyo. Here the tap root turned and extended out under the bed of the arroyo a distance of 100 in., branching freely. The horizontal part of the root was within 2 in. of the surface and bore numerous fine absorptive roots. It is evident that the unusual development of this root system is a response to moisture conditions, and it is doubtless to this ability to respond to varying conditions that

the plant owes its success as a ruderal in so many associations (figs. 29, 30).

Atriplex canescens.—This evergreen shrub is common on the fans at the mouths of arroyos and less so along the sides of the smaller arroyos. A small plant 18 in. in height and growing in a small arroyo was selected for excavation. The upper 15 in. of the soil was sandy adobe, underlaid by 32 in. of coarse sand and gravel and 15 in. of hardpan. The plant had a strong tap root which forked at a depth of 32 in. One branch continued vertically downward and penetrated the hardpan layer. The other branch

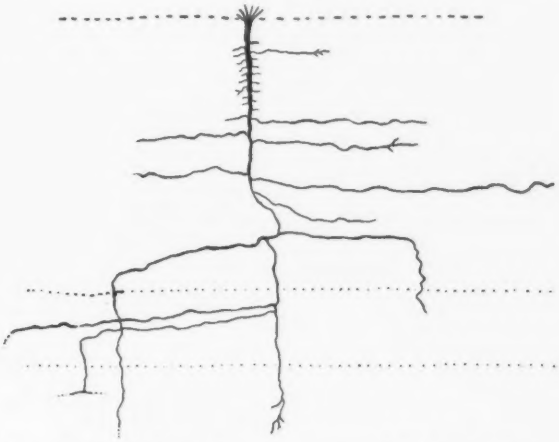


FIG. 28.—Vertical extension of root system of *Eriogonum effusum*

again forked and the subdivisions followed a tortuous course on top of the hardpan layer, twisting in every direction. One finally penetrated it diagonally. The effect of the hardpan in resisting root penetration was evident from the twisted character of the roots (figs. 31, 32, 33).

Lycium pallidum.—This solanaceous shrub is one of the most common plants along the sides of arroyos, especially where erosion is active. The root system is so peculiar that a large number of plants was examined. Unlike the roots of most of the plants of arroyo sides, the root system of *Lycium* is almost entirely horizontal.

A few laterals may extend upward. The main root of a specimen 18 in. in height was followed along the face of a steep bank for a distance of 15 ft., at which point the root was a quarter of an inch in diameter. The superficial character of the root system makes possible the vegetative multiplication by which the plant maintains itself in its unstable habitat. Erosion exposes the roots, which put forth new shoots.

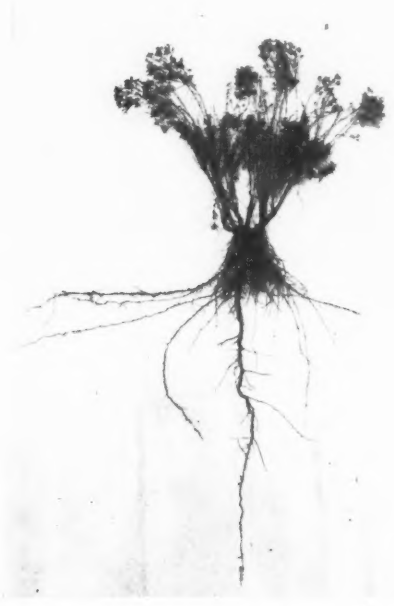


FIG. 29.—*Gutierrezia Sarothrae* from near bottom of arroyo.

Discussion

On account of the fact that here, where the soil is of fluviatile origin, the conditions to which roots are exposed vary so much, even within the habitat of a single plant, the causes of any variations in the root systems are difficult to determine. Variations are common, but they may be due to one or more of a large number of soil factors, such as the composition of the soil, its penetrability, its alkalinity, its wilting coefficient, etc. The problem of the causes of root variation is one to be attacked under laboratory conditions, in which one factor can be varied at a time.

Observation, however, made apparent the effect of at least two factors, penetrability and water content. Roots were often seen to turn abruptly from a layer of clay or adobe and follow a thin layer of sand or fine gravel containing much less water but more easily penetrable. This is illustrated by the root system of *Solanum*

eleagnifolium, shown in fig. 11. A layer of soil difficult of penetration may cause much distortion of roots entering it, as seen in the diagram illustrating the roots of *Atriplex canescens*.

The most striking instance of the effect of a variation in the water content of the soil is shown in the roots of plants growing along arroyos. Nearly all of these are characterized by long tap roots, the length of which apparently is determined by the height of the base of the plant above the moister soil below the level of the

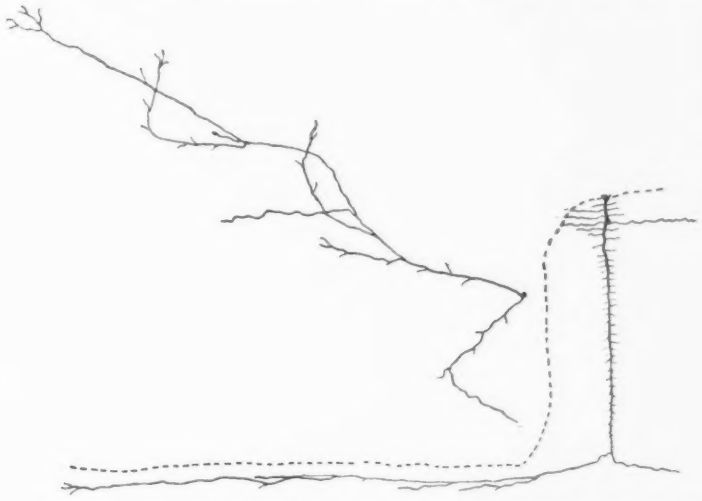


FIG. 30.—Vertical and horizontal extensions of root system of *Gutierrezia Sarothrae* from top of arroyo side.

arroyo bed. These variations are shown in the diagrams of the roots of *Ephedra*, *Gutierrezia*, *Berlandiera*, and others.

To which of the three general types of root systems that of a given individual belongs seems to be largely predetermined, a constant character of the species; but wide variations within the types, affecting the size and proportions of the root system, may occur through the influence of soil factors.

CANNON (*loc. cit.*) has pointed out the relation between the type of root system and the distribution of a species. In general, he finds that the species with root systems of the general type have

the widest distribution, while those with the specialized types are confined to peculiar habitats.

Gutierrezia Sarothrae, which has a generalized root system, is very widely distributed in primitive growths and as a ruderal. A number of species are confined here to the sides of arroyo beds and are characterized by prominent tap roots. *Lycium pallidum* has prominent horizontal roots and is confined almost entirely to rapidly eroding banks.

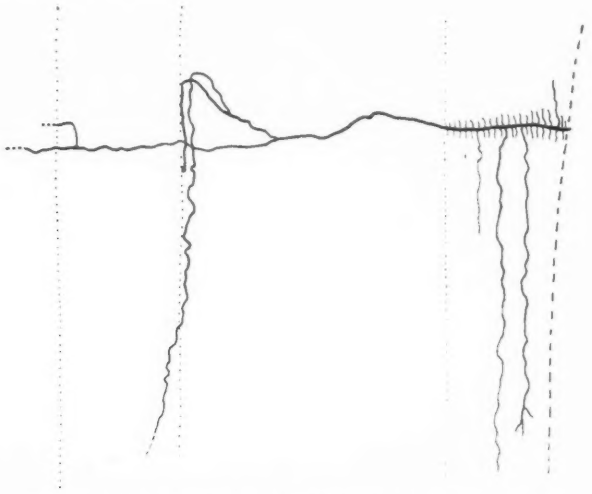


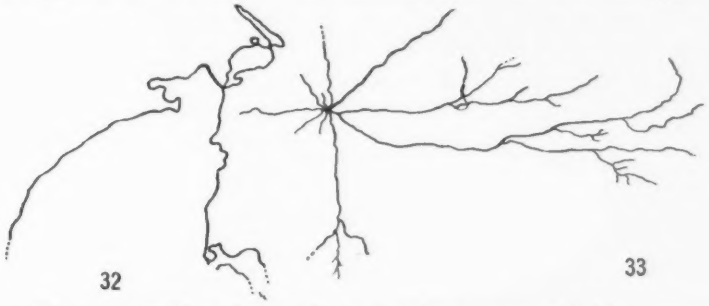
FIG. 31.—Vertical extension of root system of *Atriplex canescens*

Contrary to what might logically be expected, the species having the most fleshy roots grow in situations better watered than the average. *Cucurbita foetidissima* grows near irrigation ditches and along arroyos; *Rumex hymenosepalus* occurs in the broad arroyos crossing the mesa; *Berlandiera* is confined almost entirely to the sides of arroyo beds; *Yucca* occurs more commonly along arroyos than on the level mesa.

A knowledge of the interrelations of the roots of the plants of an association doubtless would throw light on many ecological questions. In a mesophytic association especially, the development of layers of the aerial parts of plants with reference to light

is very prominent. A comparable adjustment of the roots of plants with reference to soil moisture probably exists in all types of associations, but especially in arid habitats soil moisture is a limiting factor and root competition is more severe. Observations of the distribution of the roots of some of the associations were made on rapidly eroding arroyo banks and sand and gravel pits.

In the *Bouteloua* association, the most superficial layer of roots is that formed by the grasses, principal among which are *Bouteloua eriopoda* and *Hilaria Jamesii*. Most of the roots of these grasses occupy the upper 2 in. of soil, although some of them go much deeper. The roots of *Hilaria* are very tough and woody and reach a length of 6 ft. or more. The thorough permeation of the upper



FIGS. 32, 33.—Fig. 32, horizontal extension of root system of *Atriplex canescens* at depth of 44 in.; fig. 33, horizontal extension of superficial roots of *Atriplex canescens*.

layers of the soil by the roots of these grasses no doubt accounts for the relatively pure growth of grasses in this association. They so thoroughly remove the water from the superficial layer that seedlings of deeper rooted plants perish before the lower, moister layers are penetrated. Over large areas, overgrazing has destroyed most of the grass, giving opportunity for the entrance of such ruderals as *Gutierrezia* and *Salsola*.

In the *Dysodia-Ephedra* association, the upper layer of roots is made up of the roots of annuals, cacti, and grasses, such as *Bouteloua eriopoda, Hilaria Jamesii, Munroa squarrosa*, and *Pappophorum Wrightii*. These are very superficial and interfere little with the roots of the other plants of the association, as the grasses nowhere occupy large areas and the germination of seedlings is not prevented.

A second region of root penetration is occupied by the relatively superficial laterals so common among the plants of the region. These in general are more deeply placed than the roots of the grasses and annuals. *Dysodia acerosum*, *Aplopappus*, *Euphorbia*, and *Hymenopappus* are the principal plants.

A third layer of roots is made up of the lateral roots of *Ephedra* and the deeper parts of the root systems of *Dysodia*, *Allocarya Jamesii*, *Pachylophus hirsutus*, *Melampodium*, and others.

The fourth layer probably does not always occur, but near an arroyo it may contain more roots than any except the superficial layer. Here occur the ultimate branches of the tap root of *Ephedra*, *Gaura coccinea*, *Berlandiera lyrata*, *Stephanomeria runcinata*, and others.

The zonation of the roots reduces competition and permits the growth of a larger number of species. The root systems of the two dominant plants compete but little, since the principal absorptive roots of *Dysodia* occur in the third layer and those of *Ephedra* in the third and fourth layers. This no doubt accounts for the joint dominance of the two plants.

Summary

The region at Albuquerque differs from that of Tucson in having about two-thirds as much rainfall and much lower winter temperatures. The soil of the mesa is fluviatile in origin and very diverse in composition. The hardpan layer prominent at Tucson is not well developed. The winter annuals and the larger shrubs and cacti are absent. Most of the plants are perennial herbs.

The root systems generally penetrate rather deeply, but often have prominent laterals near the surface of the soil. The cacti and a few of the shrubs have a very superficial root system. The larger cacti show a differentiation into anchorage and absorptive roots. The plants of arroyo sides have prominent tap roots varying in length with the height of the plant above the bottom of the arroyo. Storage roots are uncommon and are more characteristic of the moister situations. Vegetative reproduction from roots is common in the plants of unstable soil.

While the causes of root variation can be accurately determined only under laboratory conditions, two factors exert a very evident influence, variation in the penetrability of the soil and in its moisture content.

The roots of the plants of an association are grouped into rather definite layers, so that root competition is lessened. The composition of an association is probably determined largely by root competition.

The writer wishes to express his appreciation of the assistance of Professor HENRY C. COWLES during the course of this investigation.

EARLHAM COLLEGE
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DEVELOPMENT OF SOME SPECIES OF PHOLIOTA

W. H. SAWYER, JR.

(WITH PLATES XVI–XX)

The taxonomy of the Agaricaceae at the present time is based upon characters of the mature plant which in many cases are slight and superficial and of uncertain homology. It is very probable that a knowledge of the origin and method of development of the different structures composing the mature fruit body would aid greatly in determining true relationships among the different genera and species, and it is for this reason that a comparative study of the morphology and development of the basidiocarp is important.

The first serious study in the Agaricaceae of the origin and differentiation of the parts of the young fruit body began over half a century ago, when HOFFMANN (**19**), in 1856, briefly described the origin of the hymenium in *Agaricus campestris* and two other species. Four years later HOFFMANN (**20**) gave a brief account of the development of several additional species, in all of which the hymenium was exogenous in origin except one, *Marasmius oreades*.

In 1866 DEBARY (**13**) studied the development of several species of Agaricaceae, and his work was followed in 1874 by HARTIG'S (**18**) description of *Armillaria mellea*, BREFELD'S (**12**) work upon *Coprinus* in 1877, and in 1889 by FAYOD'S (**15**) very cursory study of 43 species, with hymenium both exogenous and endogenous in origin. Nothing more was done along this line of research until 1906, when ATKINSON (**2**) published a thorough description of the development of *Agaricus campestris*, and the stimulus given by this work has been evidenced during the last 10 years by several publications on the development of different agarics, notably those of ALLEN (**1**), BEER (**11**), and FISCHER (**16**).

The material for the following investigation was collected during the late summer of 1915 in the vicinity of Seventh Lake, in the Adirondack Mountains, New York. The 3 species, *Pholiota squarrosa*, *P. flammans*, and *P. adiposa*, were all growing upon

rotten coniferous wood, presumably *Picea rubra*. Material of all 3 species was very abundant in several different localities, and in each case the young stages selected were identified beyond the possibility of a doubt by mature specimens associated with them. An abundance of material in all stages of growth was fixed in Carnoy's fluid and carried into cedar oil before returning to Ithaca, where it was imbedded, some in paraffin and some in collodion, and sectioned for study.

Pholiota squarrosa

Young fruit bodies, in the stage of development shown in fig. 1, are elliptical or elongate in outline and composed of hyphae loosely interwoven in the basal region, but more compact toward the apex, with some of the threads radiating from the summit. Scattered through the tissue of the fruit bodies are hyphal threads, somewhat straighter and more even in diameter than the ordinary hyphae, which are conspicuous because of their property of taking a very deep stain. These differentiated hyphae are present not only in the youngest basidiocarps, but in successive stages of development, and in all 3 species studied. Their function is unknown, but probably they serve some special purpose in nutrition. Glycogen has been shown to occur in large quantities in many fungi (*Phallus*, et al.) as a reserve food material, utilized during growth, and it may be that these peculiar threads owe their deep-staining properties to the presence of this substance.

Differentiation of stem fundament.—In the fruit body shown in fig. 1 a small, deeply staining area occurs in the central apical part; this is a region of active growth, with hyphae slightly more slender and richer in protoplasm than the other hyphae of the fruit body. This region marks the apex of the stem fundament, and in younger stages of development than the one represented here would in all probability be found to originate in the base of the fruit body, as shown in fig. 25 for *P. flammans*. In this species, as will be described more fully later, the fundament of the stem appears first in the base of the fruit body as a deeply staining area, which by progressive growth and differentiation moves to the apical part (fig. 26). A similar origin of the stem fundament has been

described in *Lepiota cristata* and *L. seminuda* (10), in species of *Cortinarius* (14), and in *Rozites gongylophora* (22).

In further stages of development, the hyphae in the stem fundament, by interstitial growth, form a compact, broadly conical area, whose apex is the dark-stained region and whose sides in median longitudinal sections slope outward at a strong angle (fig. 8). The hyphae pursue a rather uniformly longitudinal direction of growth, and are rich in protoplasm; the peripheral threads, because of this longitudinal arrangement and their deeply staining qualities, delimit the surface of the stem from the enveloping ground tissue, whose hyphae are poor in protoplasmic content and without definite direction.

DIFFERENTIATION OF HYMENOPHORE AND PILEUS PRIMORDIA.—During an early stage in the differentiation of the stem fundament there appears, in median longitudinal sections, in the ground tissue on either side of the apical part of the fundament, a small mass of hyphae, which is readily distinguishable from the surrounding tissue because of the compact nature of the hyphal complex and its property of taking a deep stain (figs. 4, 7). Serial longitudinal sections show that these hyphae occur in a ring around the apex of the stem primordium; they are the earliest evidence of the differentiation of the primordium of the hymenophore. The appearance of the hymenophore primordium differentiates the fundament of the pileus from the stem fundament, although as yet the tissue composing it is very loose and hardly to be distinguished from the surrounding ground tissue. The individual hyphae that make up the hymenophore primordium grow down from this area; at first they are crowded, very rich in protoplasm, and run in every direction, or in some cases may even be wound up into a ball (fig. 6). As the development of the fruit body proceeds, the hyphae grow out of the tangled condition in which they exist in the youngest stages of the hymenophore primordium and take a downward course. By the intercalary growth of new hyphae from above the primordium of the hymenophore becomes more compact and broadens out, keeping pace with the margin of the pileus primordium, which by interstitial and marginal growth is continually moving centrifugally toward the periphery of the fruit body. At first the hyphal threads

in the hymenophore are slender and somewhat pointed; they show a tendency to aggregate themselves at the tips into groups or tufts, with the ends of several hyphae in each tuft, and the different tufts separated by narrow interstices, so that the primordium often presents a rough and jagged appearance in this stage of its development.

BLEMATOGEN.—In the youngest fruit body sectioned (fig. 1) the universal veil consists of hyphae which push up at the apex and turn downward in all directions. There is little doubt that if younger stages had been available for study, a condition would have been found similar to that in the very young fruit bodies of *P. flammans*, where the hyphae in the beginning are loose and radiate from all over the surface of the basidiocarp (fig. 24). In the stage shown here, however, the development has proceeded to a condition where the hyphae of the lateral surface of the fruit body have taken on a direction of growth parallel to the axis of the stem fundament; a central core or strand of hyphal threads in the apex grow upward more rapidly than the surrounding tissue, and by curving backward and downward form a covering, which is the blematogen, over the entire surface of the fruit body. The hyphal cells thus exposed upon the outside become enlarged and many of the outermost ones lose their protoplasmic content and become oval or globose. The hyphae composing the fruit bodies shown in fig. 1 are 3–5 μ in diameter near the base, and in the apical region they are slightly more slender, averaging about 3 μ in thickness, while the hyphae of the blematogen layer are much thicker, being 8–15 μ in diameter. The condition existing here is somewhat similar to the origin of the blematogen in *Coprinus lagopus* (12), as described and figured by BREFELD. In this plant, according to BREFELD, before pileus formation, the fruit body consists simply of the stem primordium, whose outer hyphae have free ends, and do not enter into the formation of the stem; the pileus primordium is differentiated at the apex "upon the point of the stem," and from this pileus primordium hyphae grow out and turn downward in all directions, forming the "pileus-volva." In the meantime the loose peripheral threads of the stem primordium, the "stem-volva," grow upward to meet these downward growing hyphae from the

apex, and the two groups of threads grow into each other and intermingle to form the common mass of the "volva." In *P. squarrosa* there is a material difference from the condition just described for *C. lagopus*, since in this case there are no hyphae on the stem surface which grow upward and unite with the downward growing threads. Figs. 2, 3, and 7 show the central strand of hyphae just mentioned; in fig. 5 the character of the blematogen hyphae may be seen.

ORGANIZATION OF PILEUS.—Coincident with an early stage in the development of the hymenophore primordium, median longitudinal sections show that the fundament of the pileus is becoming differentiated from the surrounding tissue (fig. 8). The hyphae become richer in protoplasm and by interstitial growth form a more compact structure. This organization proceeds from the center outward in a centrifugal manner, the margin of the pileus keeping pace with, and contributing to, the growth of the hymenophore primordium. During the early stages of differentiation of the pileus some of the hyphae arise from the stem, but its later growth is probably due entirely to interstitial and marginal increase of its own elements, which are interwoven in all directions, thus differing from the hyphae of the stem, which in general run parallel to the stem axis. The pileus elements merge gradually with the blematogen and there is no sharp line of division between the two structures. The cells of the blematogen hyphae, however, are swollen and have thick walls, which stain deeply, while the pileus hyphae are slender and do not take a deep stain after the pileus is well organized, so that a general distinction is evident. In addition, the peripheral threads of the pileus, composing the "cortex," are very compact and form a dark line between the pileus and blematogen, as shown in fig. 13.

FORMATION OF PALISADE LAYER.—Following the stage when the hyphae are arranged in irregular tufts, the hymenophore primordium becomes more compact (fig. 12). The ends of the individual hyphae grow down to nearly the same level and become clavate. This even area is the palisade layer. Serial sections show that it is formed first around the stem, while the hymenophore near the pileus margin is still in the uneven, jagged stage. The

pileus margin at this time is turned downward and often somewhat incurved as a result of epinastic growth, and lies nearly parallel with the surface of the stem, which still slopes outward at a slight angle (fig. 10).

FORMATION OF ANNULAR PRELAMELLAR CAVITY.—During differentiation of the young basidiocarp some ground tissue is left below the hymenophore primordium in the angle formed by the junction of the stem and pileus fundaments. In later development this ground tissue increases to some extent by interstitial growth, but the more rapid growth of the stem, hymenophore, and pileus subjects it to tension, and it very early becomes loose in texture (fig. 4). As the stem elongates and the pileus broadens out, this tension is further increased, so that the ground tissue becomes still looser, with large spaces between the hyphae. At first it only partially tears away from the surface of the hymenophore, and as a result the gill cavity thus formed is weak, with strands of ground tissue traversing it (figs. 13, 14, 16–20). The strength of the prelamellar cavity varies in different individuals, as has been shown to be the case in *Agaricus rodmani* (**8**); but in any case, in later stages, but long before the gills are exposed by rupture of the veil, the strands of ground tissue become completely broken away, and the edges of the lamellae are entirely free within the gill cavity.

ORGANIZATION OF PARTIAL VEIL.—The terms "blematogen" or "universal veil" and "marginal" or "partial veil" have been interpreted by ATKINSON (**5**), and are used in the same sense here. The formation of the blematogen has already been described. The radial growth of hyphae in the apex of the young fruit body is very rapid for a time, and a thick layer is formed, enveloping the entire plant, but it is more dense in the apical region (figs 7–9). This rapid growth soon ceases, however, the hyphae become thick-walled and poor in protoplasmic content, the peripheral ones die, and become enlarged and swollen. Because its growth has nearly ceased, the universal veil becomes subject to tension from the expansion of the parts within and breaks up into the large, conspicuous, squarrose scales (fig. 13) which cover the stem and pileus and give the plant its specific name. The partial veil has its origin in

the ground tissue left in the angle between the stem and hymenophore. This tissue increases, both by interstitial growth and by the addition of hyphae which grow down from the pileus margin (fig. 22). By the time that the gills are well formed this tissue occupies a considerable area lying between the margin of the pileus and the surface of the stem, and forming the floor of the gill cavity. It is covered externally by the blematogen, with the inner surface of which its hyphae are interlaced, as some of them are with the stem surface. When expansion of the pileus occurs and the veil is ruptured, it is left upon the stem as an annulate membrane composed of two layers, the coarse, scaly blematogen layer below and the partial veil above.

ORIGIN AND DEVELOPMENT OF LAMELLAE.—In a recent publication ATKINSON (9) has shown that in the Agaricaceae thus far studied there are two types in regard to the origin and development of the lamellae. First, the "*Agaricus*" type, in which the gills arise by downward growing radial salients of the hymenophore, accompanied or preceded by a more or less well developed annular prelamellar cavity. Second, the "*Amanita*" type, in which there is no general annular prelamellar cavity, and the origin of the lamellae is a series of trabeculae extending from the pileus fundament to the stem, and attached to both. *P. squarrosa* obviously belongs to the first type of development, since we have already seen that in the course of development of the young fruit body a general annular, prelamellar cavity, though weak, and a palisade layer are formed.

The origin and differentiation of the gills from the hymenophore is centrifugal; the later formed and younger parts are nearer the margin, becoming progressively older as the stem is approached. Successive stages in the origin and development of the lamellae, therefore, may be studied by means of serial longitudinal sections, commencing at the pileus margin and going toward the center of the fruit body.

Figs. 16–21 represent such a series of sections. The basidiocarp from which they were made was one selected from material imbedded in collodion, and the sections, 15–20 μ thick, were cut with the sliding microtome. The use of collodion (or celloidin) as

an imbedding material obviates the difficulty sometimes met with in the use of paraffin, that delicate structures may be deformed or dislocated by the heat of the oven or in spreading the paraffin ribbons. Furthermore, the cutting of thicker sections, with a sliding stroke, offers little chance for the displacement of structures, which might happen in cutting thin paraffin sections. I mention these points because some might suspect that the tearing away of the ground tissue below the hymenophore, as shown in the following figures, might be due to manipulation of the tissue, but such is not the case.

Fig. 16 represents a section near the margin of the pileus; the hyphae of the hymenophore are growing down in little tufts, and at this time present a very loose, uneven surface. A considerable number of hyphae from the ground tissue below may be seen spanning the prelamellar cavity and united indiscriminately with the downward growing tufts of the hymenophore and with the hyphae in the spaces between them.

In fig. 17, from a section a little nearer the stem, the hymenophore on either side of the section presents the same loose, uneven surface as in the preceding figure; but in the middle the hyphae have enlarged at the tips and become blunt, and the ends have grown down to form an even surface, the palisade, from which the ground tissue is almost entirely broken away. The reason that the palisade is in the middle of the section, with undifferentiated tissue on either side, is readily seen, if we remember that the hymenophore is in the form of a circle around the stem apex, so that tangential sections, in passing toward the center, cut through older parts of the hymenophore in the middle of the section, with younger parts on either side. For further details see the papers on *Agaricus rodmani* and *Coprinus* species by ATKINSON (**8, 9**), where, by means of diagrams, the orientation of the parts of the fruit body in relation to longitudinal sections is made very clear.

In fig. 18, in addition to the undifferentiated tissue on either side and the palisade layer, two slightly downward projecting folds of the latter may be seen. These slight downward projections of the palisade layer represent the first origin of the lamellae in this area. To one a strand of ground tissue is attached, while the other

is completely free. That this ground tissue has nothing to do with the formation of the palisade is shown by the fact that, as already stated, it is attached indifferently to the hymenophore primordium, and in many cases is largely broken away from the tufts and between them before the palisade layer is formed. Furthermore, these tufts of the hymenophore primordium are not the primordia of the lamellae, since before the origin of the latter they become lost in the even palisade (figs. 17, 18). In those instances where the ground tissue remains adherent to the edges of the lamellae for some time, and not to the palisade between them, it is due to the fact that through the downward growth of the gills the strands of hyphae attached to their edges are subject to less strain than the hyphae attached between them, and so keep their attachment longer.

The downward growth of the lamellae may be partly initiated by the pressure in the palisade layer due to the rapid growth and enlargement of its hyphae, which would produce a tendency to throw the palisade into folds. The chief agency in their formation, however, seems to be the downward growth of radially arranged groups of hyphae in the hymenophore, which are very active in growth at this time, as indicated by their deep stain. These radial lines of deeply staining hyphae push down into the folds of the palisade and form the trama of the gills (figs. 19–21). The further growth of the lamellae in depth takes place by apical and interstitial growth in the trama. Later stages in development (fig. 23) show the hyphae from the trama turning outward on all sides to add to the growth of the palisade layer of the gills.

Pholiota flammans

PRIMORDIUM OF BASIDIOCARP.—The very young fruit body, before any internal differentiation has taken place, is a compact structure, composed of slender, intricately interwoven hyphae, 2–3 μ in diameter, and rich in protoplasm. The hyphae have a general direction of growth away from the substratum, and many free hyphal ends radiate outward all over the surface of the basidiocarp, so that the peripheral area is somewhat looser in structure than the compact central region (fig. 24). This loose radiating zone of hyphae upon the outside represents an early stage in the

development of the blematogen, which is probably present from the first appearance of the fruit body primordium.

DIFFERENTIATION OF STEM FUNDAMENT.—As development proceeds, the hyphae in the base of the primordium take on more active growth than the others, and by interstitial increase form a very dense structure. This new area of growth, which is the stem fundament, is shown as a deeply stained region in the base of the fruit body in fig. 25. As growth continues, the cone-shaped stem fundament advances toward the apex of the fruit body. In fig. 26 the most deeply stained portion represents the rapidly growing, progressive apex of the stem fundament; the more compact tissue below represents its earlier differentiated base; and the outer zone of loose tissue surrounding the whole is the blematogen.

DIFFERENTIATION OF HYMENOPHORE AND PILEUS PRIMORDIA.—The first evidence of the hymenophore primordium is the appearance of a ring of compact, slender hyphae which surrounds the upper part of the stem fundament and grows down into the ground tissue, clothing the latter. The appearance of these differentiated hyphae marks off the pileus area from the stem fundament. In some cases the pileus fundament probably exists before the appearance of the hymenophore primordium, as indicated by the slight divergence of the hyphae from the apex of the stem fundament or by the more rapid growth in the region of the future pileus; but no sharp distinction can be drawn between the fundaments of pileus and stipe until the primordium of the hymenophore is differentiated. An early stage in the development of the latter is shown in fig. 30. As development continues, the pileus and hymenophore progress together in growth in a centrifugal manner. New elements from the pileus margin contribute to the hymenophore primordium, which also increases by interstitial growth. Interstitial growth also takes place in the pileus primordium, making it more compact. In the stage of figs. 31 and 32 the stem and pileus are well organized and the hymenophore has become compact, with uneven surface. The palisade is formed later, as shown in figs. 34 and 37. The hyphae are not as crowded, or so conspicuously clavate, as in *P. squarrosa*.

BLEMATOGEN.—The blematogen is present from the first appearance of the fruit body primordium. During early development

it radiates from all parts of the surface as a loose aggregation of hyphae with numerous interhyphal spaces (fig. 29). The new elements arise chiefly in the apex of the young fruit body and extend outward in a radial direction, curving backward as the fundament of the basidiocarp elongates and as the stem and pileus primordia are differentiated. This peculiarity of the blematogen is like that in *P. squarrosa*. This downward growth continues until a thick covering is formed (fig. 31). The elements of the blematogen are thick-walled and somewhat larger than the other hyphae of the basidiocarp, but they are not conspicuously globose, as in *P. squarrosa*.

Early in its formation some of the threads begin to break down and gelatinize, and the universal veil soon becomes a gelatinous matrix, imbedded in which may be recognized the remnants of hyphae not yet disorganized. Such a condition exists in fig. 31, and a high magnification (fig. 38) shows a sharp contrast between the gelatinized blematogen and the cortex of the pileus at this stage. A very similar condition of the universal veil has been noted in *Stropharia ambigua* by ZELLER (**25**).

The disorganization of the blematogen elements does not go beyond the degree shown in fig. 38. Sections perpendicular to the pileus in mature specimens show a very similar condition; there is a gelatinous ground substance filled with dead hyphae whose general course is parallel to the surface of the pileus. Through drying of the outer part and the tension exerted upon it by the expanding pileus the blematogen breaks up into scales. These scales differ very markedly, however, from the stout, pointed scales of *P. squarrosa;* they are thin and delicate and more or less fibrillose in nature.

The gelatinization takes place only over the pileus, the blematogen clothing the stem very nearly resembling that of *P. squarrosa*. For this reason the scales covering the pileus in the mature plant are more delicate than those covering the stem, as indicated in the description of *P. flammans* in SACCARDO'S *Sylloge* (**23**), in which it is stated that the pileus is covered with superficial scales, while the stem is scaly-squarrose.

FORMATION OF ANNULAR GILL CAVITY AND MARGINAL VEIL.—The formation of the gill cavity agrees in all essentials with its

formation in the preceding species. The ground tissue is loose from a very early stage, and through expansion of the different parts of the fruit body it becomes torn away from the lower surface of the hymenophore. This separation from the hymenophore is completed at an earlier stage than in *P. squarrosa*, and consequently a well defined cavity is present before the origin of the lamellae (figs. 34, 37).

During the development of the hymenophore primordium and the breaking away of the ground tissue below hyphae are growing down from the pileus margin. These threads penetrate the ground tissue below the prelamellar cavity and mingle with those on the surface of the stem. In fig. 33 they may be seen curving inward from the pileus margin. In the stage represented here they have not yet reached the surface of the stem, and the loose ground tissue surrounding the latter may still be seen between it and the advancing hyphae from the pileus margin. These threads are sharply contrasted with the other tissue of the basidiocarp because of their short cells, which in some cases are as broad as they are long. In addition, they may be distinguished from the blematogen external to them by their deeper stain. These hyphae from the pileus margin, together with the ground tissue below the hymenophore, form the partial veil; it tears away from the stem at an early stage in the expansion of the plant, and may in some cases hang down from the pileus margin as a thin appendiculate veil.

ORIGIN AND DEVELOPMENT OF LAMELLAE.—The origin and development of the gills in this plant does not differ materially from the condition already described for *P. squarrosa*. Here we have a better defined gill cavity, with no ground tissue traversing it and connecting with the palisade layer. The latter is composed of hyphae with blunt ends, standing in an even surface, compact, but not crowded. Serial longitudinal sections, from the tangential to the median, show that the formation of the annular gill cavity, of the hymenophore, and the development of the lamellae, all proceed by centrifugal growth; that is, the older and first formed parts of these structures lie near the stem and progress by differentiation and growth toward the periphery of the fruit body. As has already

been stated, the hyphae in the palisade layer are not crowded enough to produce any great pressure, and it would seem that the origin of the gill salients, which appear as downward folds of the palisade, is due entirely to the growth and elongation of radial lines of hyphae in the hymenophore, which push the palisade down in folds, the young gills, as described for *Hypholoma sublateritium* (1) and *Stropharia ambigua* (25). The gill salients are broader than in *P. squarrosa*, and this may be due to the fact that when thrown into folds by downward growth of the hyphae above, because of the less crowded condition of the palisade, they are not subjected to as great lateral pressure as in that species.

Fig. 39 is from a tangential longitudinal section of a basidiocarp with sterile gills, that is, the palisade layer has failed to form. The cystidia, which develop from the trama of the gill, are very noticeable as deeply staining clavate bodies. The situation presented here is interesting because of its bearing on the question recently raised by LEVINE (21) in regard to the origin of the lamellae in the Agaricaceae. The points of growth for the origin of the lamellae, as described by ATKINSON in several species of the Agaricaceae, including *Agaricus rodmani* (8) and *Coprinus comatus*, *C. atramentarius*, and *C. micaceus* (9), occur in radial areas of hyphae in the hymenophore, which develop centrifugally and grow down more rapidly than the other hyphae. These areas are the gill tramae, and push the palisade into regularly spaced folds, which are the salients of the lamellae themselves. This method of origin of the lamellae occurs in the three species described here.

According to LEVINE'S conception, radiating ridges of palisade cells arise in the fundamental tissue, and by continued differentiation and downward growth of new palisade cells, split apart. The adjacent halves of neighboring ridges then come together and unite to form a lamella. The trama of the gill would thus be formed by the coming together of preexisting palisade cells from adjacent ridges. If no palisade cells were differentiated, therefore, no trama could be formed. In this case, however, no palisade cells are formed, yet the trama of the gill and the cystidia, which come from trama tissue, develop normally.

Pholiota adiposa

PRIMORDIUM OF BASIDIOCARP.—In the youngest fruit body sectioned the mycelial threads grow out from the substratum to form a compact mass of hyphae which are closely interwoven and run in all directions. From this structure hyphae gradually assume an upward direction of growth, forming a papilla-like projection (fig. 42), the fruit body primordium. The threads in the basal mycelium from which the fruit body arises are very uneven in size; those of the primordium are even in size, with free ends radiating out all over the surface.

STEM FUNDAMENT.—The stem fundament probably differentiates first in the base of the fruit body, as in the preceding species. In fig. 43 its apex appears as a compact, dark-staining region near the top of the basidiocarp, surrounded by the looser tissue of the young blematogen. The fundament hyphae are very slender at first, dense in protoplasm, and closely intermingled, running in all directions.

PRIMORDIA OF HYMENOPHORE AND PILEUS.—When the hymenophore and pileus primordia appear, the stem has become well organized as a compact conical area, surrounded by the loose universal veil. The first differentiation of the pileus fundament becomes evident through the growth of hyphae upward from the stem apex; these spread outward laterally, so that at this stage the stem and pileus areas together resemble a sheaf of wheat. At the same time some of these hyphae become subject to strong epinastic growth, and curve down in a ring around the stem apex, forming the primordium of the hymenophore, which definitely differentiates the pileus area from the stem fundament. The growth of the pileus continues by interstitial and marginal increase of its elements, and the hymenophore broadens out by growth within itself and by intercalary growth from the pileus margin, with which it keeps pace. The hyphae of the hymenophore primordium often become aggregated into tufts (fig. 48), as in *P. squarrosa*. Ground tissue may still remain attached to these tufts. The hyphae now grow down to the same general level, their ends become blunt, and form an even surface. During early development the hymenophore extends down on the stem farther than in the two preceding

species (figs. 45, 47). In later stages it loses this decurrent character.

BLEMATOGEN.—The universal veil exists from the beginning; its development proceeds very much as in *P. flammans*. At first its hyphae radiate from all over the surface of the basidiocarp. Later the growth of new elements is largely confined to the apex. The peripheral cells become enlarged, thick-walled, with a diminution in protoplasmic content (fig. 52). The outer ones appear empty and dead. Gelatinization takes place here, as in *P. flammans*, but later, after the gills are well formed. Sections through the mature pileus show that the blematogen has a structure very comparable in the two species, in either case composed of a structureless matrix in which are imbedded dead hyphae, with a general course parallel to the pileus surface. At first disorganization occurs only over the pileus, but in the mature plant the gelatinization takes place over the entire surface.

The mature pileus in *P. flammans* is dry, and in *P. adiposa* is gelatinous or viscid. This difference is due to the fact that in the latter species the disorganization of the blematogen elements proceeds farther than in the former, so that the walls of the hyphae become more gelatinous, with a greater capacity for absorbing water. The surface of the blematogen breaks up into scales, as in *P. flammans*, but the scales are very different in character. They are not thin and fibrillose here, but in wet weather appear like little lumps of jelly on the surface and are easily lost, so that it is not uncommon to find old fruit bodies with the surface of the pileus nearly free from them, especially over the central area.

ANNULAR PRELAMELLAR CAVITY.—The gill cavity begins to develop soon after the appearance of the hymenophore and pileus primordia. The growth and expansion of these parts tear apart the ground tissue below the former, making it loose, with large spaces between the hyphae. At first, strands of hyphae span the cavity and remain attached to the hymenophore, but these have all disappeared by the time the palisade layer is formed, and the cavity is then clear (fig. 47).

MARGINAL VEIL.—The partial veil is composed of the ground tissue left in the angle between the hymenophore and stem, together

with some hyphae which grow down from the pileus margin. This growth, however, is not as strong as in *P. flammans*. The partial veil is covered externally by the blematogen. It ruptures at the pileus margin during expansion of the plant, leaving a thin and fugacious annulus on the stem.

ORIGIN AND DEVELOPMENT OF LAMELLAE.—The origin and growth of the gills take place in this species much as in *P. flammans*. The first evidence of the origin of the gill salients is the downward projection of the palisade layer in broad folds (figs. 50, 51). A single one of these broad folds includes several of the tufts which earlier appear in the hymenophore primordium and become lost in the palisade; consequently, these tufts cannot be considered as gill fundaments or directly concerned in their origin. At the apex of the folds the ends of the palisade cells may in some cases spread slightly apart, showing that considerable pressure is exerted by the downward growing hyphae from the hymenophore above. Under no circumstances, however, do the gill salients show any evidence of splitting; the hyphae merely spread slightly apart at the ends, and in later stages come together again to form an uninterrupted palisade. Serial sections show that the formation of the gill salients is radial and centrifugal.

Figs. 53, 54, and 55 show a condition that might easily lead to a wrong interpretation of the origin of the lamellae by one not familiar with the orientation of the parts involved. A similar condition existing in *Agaricus rodmani* has been explained by ATKINSON (**8**) and so will not be gone into in detail here. The sections are tangential in the margin of the pileus at a stage in development when the pileus margin is enrolled. The attachment of the gill trama both above and below does not mean that the trama of the gill has grown down and united with the tissue below, as might appear at first glance. The pileus margin, and consequently the hymenophore, is incurved so that the hymenophore lies both above and below the gill cavity, and the attachment of the trama below as well as above represents its point of origin. The direction of growth of the trama is not in the plane of the section, but perpendicular to it. A similar condition exists in *P. squarrosa* and *P. flammans* at a certain stage in the organization of the pileus before it has expanded.

Sequence of plant parts

The relative time of origin of the primordia of the basidiocarp is of some historical interest. FRIES (17), influenced perhaps by the preformation theory, still in vogue in his time, believed that all the parts, pileus, stem, and hymenophore, although indistinguishable, existed already formed in the young fruit body and unfolded simultaneously. SCHMITZ (24) held that a successive formation of new parts occurred; that the development of new parts rose upward just as gradually as in the higher plants, so that those standing higher came into evidence later than those below; and that therefore the matrix developed before the stipe, the latter before the pileus, and the latter before the hymenium. Later, FAYOD (15) formulated a general law to the effect that the first part to be differentiated is always the pileus primordium.

More recent work has shown that no general rule can be laid down as to what primordium shall have precedence in differentiation. In *Agaricus campestris* (2), *A. arvensis* (3), *A. rodmani* (8), *Armillaria mellea* (4), and *Stropharia ambigua* (25), the hymenophore primordium is differentiated first. In *Hypholoma sublateritium* (1), *H. fasciculare* (11), and *Amanitopsis vaginata* (7) the pileus area is first outlined. The formation of the stem fundament is the first differentiation to take place in *Lepiota cristata* and *L. seminuda* (10), several species of *Cortinarius* (14), *Rozites gongylophora* (22), and the 3 species of *Pholiota* described.

Even in the same species variations may occur as to the relative time of appearance of the different primordia. ATKINSON (10) has shown this to be probable in *Agaricus arvensis* (3) and *Lepiota clypeolaria* (6). In *P. flammans* it would appear that the fundament of the pileus in some cases differentiates before the hymenophore primordium, and this may be true of the other two species described. In all 3 species, however, the appearance of the stem fundament precedes the other primordia of pileus and hymenophore.

Summary

1. The basidiocarp primordium consists of slender hyphae intricately interwoven; they are arranged more loosely in the

peripheral region, and radiate from the entire surface of the fruit body.

2. The blematogen is present from the first differentiation of the fruit body primordium, and in its earliest stages consists of the loose, radiating peripheral hyphae. In subsequent growth it forms a thick layer enveloping the entire plant; in *P. flammans* and *P. adiposa* it becomes partially disorganized by gelatinization.

3. The formation of the stem fundament is the first differentiation to take place in the young fruit body. It originates in the basal part of the basidiocarp and by growth and differentiation progresses toward the apex.

4. The primordium of the hymenophore is differentiated around the apex of the stem fundament as an annular internal zone of new growth. Frequently, before the hymenophore appears, a slight divergence of hyphae from the stem apex indicates differentiation of the pileus. When the hymenophore primordium is differentiated, it marks off clearly the limit between the pileus and stem. It consists of slender hyphae, rich in protoplasm, which grow downward. At first the lower surface is uneven and loose, but by continued growth the hymenophore becomes compact and the hyphae grow down to the same level, forming an even palisade area. The growth of the hymenophore and organization of the pileus proceed centrifugally.

5. The annular prelamellar cavity is formed by the tearing away of the ground tissue from the lower surface of the hymenophore, due to the tension exerted by the growth and expansion of the plant parts. It is weak in *P. squarrosa*, and well formed in *P. flammans* and *P. adiposa*, before the origin of the lamellae.

6. The marginal veil consists of two layers; the inner portion is composed of the ground tissue left in the angle between the stem and the hymenophore after their differentiation, which increases by its own growth and by the addition to it of hyphae from the pileus margin. The outer portion is a section of the universal veil between the pileus margin and the surface of the stem.

7. The lamellae originate as a series of radiating areas of active hyphae in the hymenophore, which grow down and push the palisade layer into folds. The points of growth for the origin of the gills are

in these downward growing areas in the hymenophore, and the first folds in the palisade are the salients of the lamellae themselves. In *P. flammans* and *P. adiposa* the gill salients are very broad; in all 3 species their origin and differentiation is centrifugal and their subsequent growth is downward into the gill cavity.

In conclusion, I wish to acknowledge my deep obligation to Professor G. F. ATKINSON, under whose direction this work was done, for his unfailing interest and many helpful suggestions.

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EXPLANATION OF PLATES XVI–XX

The following microphotographs were made with the Bausch and Lomb vertical camera and Zeiss lenses, and with a horizontal Zeiss camera.

PLATES XVI, XVII

Pholiota squarrosa

FIG. 1.—Median section, showing apex of stem fundament as dark-stained area near center of figure; very deeply stained hyphae scattered through stem fundament; hyphae of blematogen radiating from summit; ×50.

FIG. 2.—Slightly older stage than above, showing central strand of hyphae that curve downward from summit to form blematogen; ×50.

FIG. 3.—Median section, showing radiation of blematogen hyphae and their loose arrangement at periphery of fruit body; ×50.

FIG. 4.—Median section at a stage closely following differentiation of hymenophore; cone-shaped stem fundament occupies lower part of figure, with hymenophore primordium on either side of deeper stained apex, showing as two small, deeply stained areas, which differentiate pileus primordium above from stem fundament; below hymenophore primordium ground tissue is becoming loose in structure; outside the whole is the thick universal veil; ×33.

FIG. 5.—Left side of same section, magnified more to show character of blematogen; at extreme left are enlarged, thick-walled, empty cells of outer

portion of blematogen; at extreme right may be seen a part of hymenophore primordium with loose ground tissue below; ×56.

FIG. 6.—Left side of median section about the time when hymenophore primordium is first differentiated; tangled, compact mass of hyphae of very young hymenophore near center, with looser ground tissue surrounding it; ×133.

FIG. 7.—Median section at about the same stage of differentiation as fig. 4; two compact, dark spots are hymenophore primordium; from summit blematogen hyphae are curving outward and downward; ×50.

FIG. 8.—Median section; conical stem fundament below; hymenophore primordium on either side, and pileus fundament appearing above hymenophore as a more compact, central area; loose ground tissue clothes stem, and outside is deep-stained blematogen; ×18.

FIG. 9.—Tangential section of same fruit body; hymenophore primordium appears as dark horizontal area, with uneven lower surface; irregular dark region just below is oblique section of stem surface; ×18.

FIG. 10.—Median section, at a stage when palisade has formed; gill cavity appears as a narrow slit almost closed because of epinastic growth of pileus margin; below, the looser ground tissue; ×133.

FIG. 11.—Tangential section of same fruit body; ground tissue is tearing away from hymenophore to form gill cavity; above hymenophore is crescent-shaped pileus, with the much thicker blematogen outside; ×33.

FIG. 12.—Higher magnification of section near preceding to show blunt ends of palisade hyphae, with loose ground tissue below; above is dense hymenophore; ×133.

FIG. 13.—Blematogen has broken into scales; stem and pileus are well organized; below hymenophore on either side is gill cavity, weak, with strands of ground tissue traversing it; dark line between pileus and hymenophore indicates area of more rapid growth; ×13.

FIG. 14.—Left side of a section similar to fig. 13, and showing same features enlarged; ×32.

FIG. 15.—Tangential section, showing compact hymenophore; no gill cavity in this section because its centrifugal development has not yet reached tangential area here shown; ×18.

FIGS. 16–20.—Serial tangential sections from pileus margin toward stem; in fig. 16 hymenophore is uneven and gill cavity is weak, with considerable ground tissue still attached to hymenophore; in fig. 17 hymenophore has become even in center, forming a palisade area; gill cavity is stronger than in preceding section; in fig. 18 two gill salients appear as folds in level palisade; figs. 19 and 20 show older stages of these and other salients nearer stem; ×32.

FIG. 21.—Higher magnification of fig. 20, showing origin of gill tramae in hymenophore as regularly spaced, deeper staining areas of rapidly growing hyphae; ×128.

FIG. 22.—Left side of median section in a stage after lamellae are well differentiated; at extreme left are hyphae growing down from the pileus margin into ground tissue below; knife has passed lengthwise through a gill, making it thin enough to look through and see level palisade between it and next gill; ×128.

FIG. 23.—Tangential section, showing gills with trama and palisade; ×148.

PLATES XVIII, XIX

Pholiota flammans

FIG. 24.—Median section of very young fruit body, which is broken just above substratum of rotten wood; loose, radiating threads represent early stage in formation of blematogen; no internal differentiation has taken place; ×60.

FIG. 25.—Stem fundament forming in base of fruit body, its apex stained very deeply; outside is blematogen; ×36.

FIG. 26.—Slightly later stage in which, by progressive growth, apex of stem fundament has advanced to summit of fruit body; ×36.

FIG. 27.—Somewhat older fruit body, with stem fundament differentiated, apex showing near summit as darker area; very deeply stained, isolated hyphae are scattered through stem fundament; ×36.

FIG. 28.—Same as fig. 27; ×36.

FIG. 29.—Older fruit body, with blematogen radiating from entire surface; stem area well organized, but no other differentiation; ×36.

FIG. 30.—Small area on either side with looser ground tissue below represents very young hymenophore; below is conical stem area and above is pileus fundament which is partially organized; over pileus area blematogen hyphae are spreading downward and have begun to gelatinize; on sides blematogen hyphae are still radiating laterally; ×20.

FIG. 31.—Stem and pileus areas well organized; hymenophore shows as very deeply stained area on either side below pileus; hyphae from pileus margin are shown growing down and uniting with stem surface; outside is lighter stained, partially gelatinized blematogen; ×36.

FIG. 32.—Tangential section of same, showing uneven stage of hymenophore, with ground tissue in gill cavity; ×36

FIG. 33.—Left side of median section; uneven surface of hymenophore primordium is shown above weak gill cavity; outside hymenophore, hyphae from pileus margin are growing in toward stem surface; majority of these hyphae have not reached stem and ground tissue is seen between them and the latter; at extreme left is blematogen; ×86.

FIG. 34.—Right side of median section at a later stage; gill cavity well formed; above it hymenophore has organized palisade near stem, but is still uneven near pileus margin; short-celled hyphae from latter are filling in ground tissue below cavity to help form partial veil; ×142.

FIG. 35.—Tangential section, showing early stage in organization of palisade by hymenophore; gill cavity below; ×125.

FIG. 36.—Tangential section very near stem, showing two broad gill salients, covered by palisade; some clavate cells of latter project beyond surface, giving rough appearance; note disorganized condition of universal veil; ×86.

FIG. 37.—Tangential section, showing even palisade and large gill cavity; ×31.

FIG. 38.—Section highly magnified to show contrast between pileus and gelatinized blematogen; ×142.

FIG. 39.—Tangential section of fruit body with sterile gills; note absence of any palisade, and the cystidia, which develop from trama tissue of gill; ×142.

FIG. 40.—Tangential section, showing normal gills at about same stage as preceding; hyphae of trama may be seen turning outward to contribute to palisade; cystidia are visible on edges of lamellae; ×142.

FIG. 41.—Tracheid of *Picea* inclosed in tissue of stipe; this fruit body was in advanced stage of development, with lamellae well differentiated; ×230.

PLATE XX

Pholiota adiposa

FIG. 42.—Median section of very young fruit body; narrow, dark area in center is a bit of substratum inclosed in tissue; threads radiating from surface form young blematogen; ×50.

FIG. 43.—Later stage, showing apex of stem fundament at summit of basidiocarp; ×56.

FIG. 44.—Still later stage in development; ground tissue is becoming loose around apex of stem fundament; blematogen hyphae have largely ceased lateral growth and are radiating out from summit of fruit body; ×33.

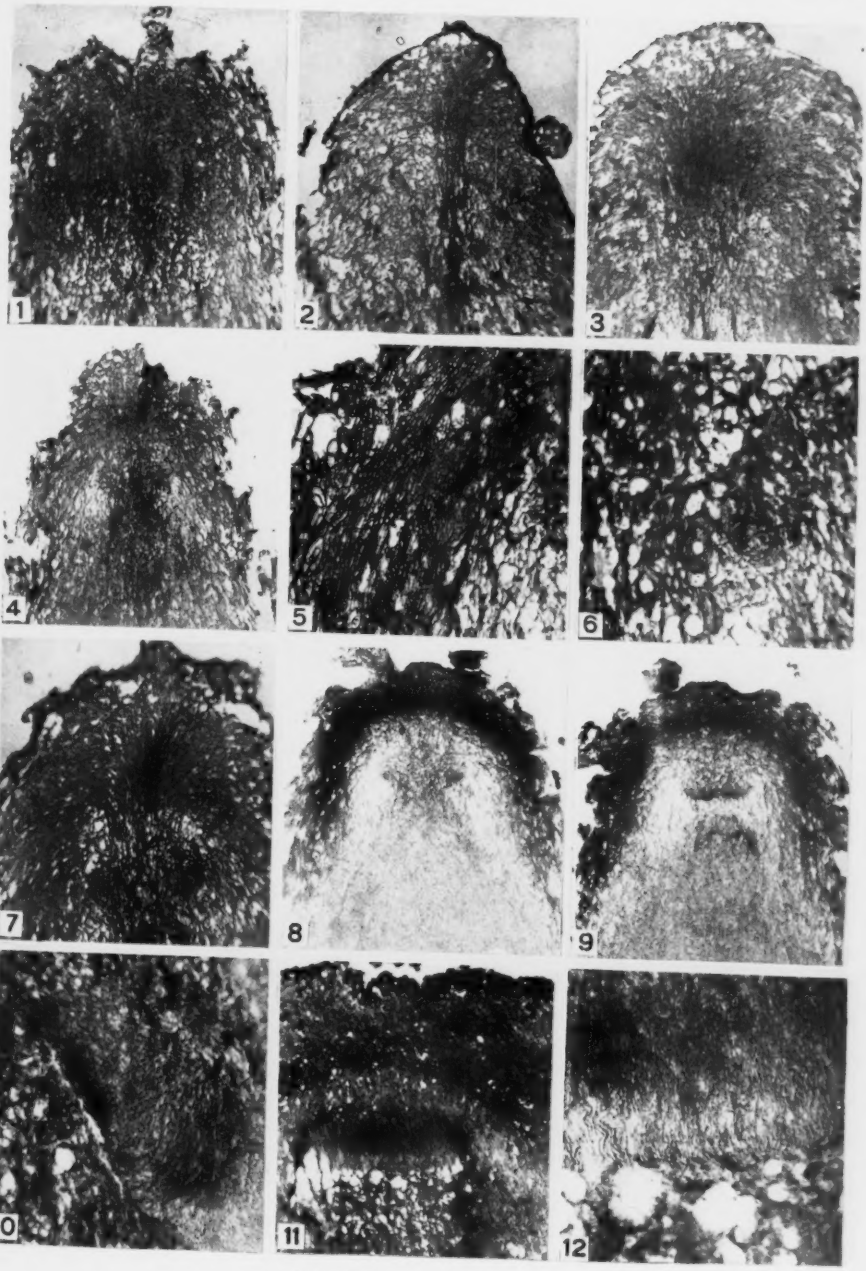
FIG. 45.—Median section of stage when stem and pileus are well organized and hymenophore is forming palisade; heavy blematogen shows no signs of disorganization that occurs later; ×18.

FIG. 46.—Tangential section at same stage in development; because it is nearer pileus margin hymenophore has not formed palisade in this section; ×18.

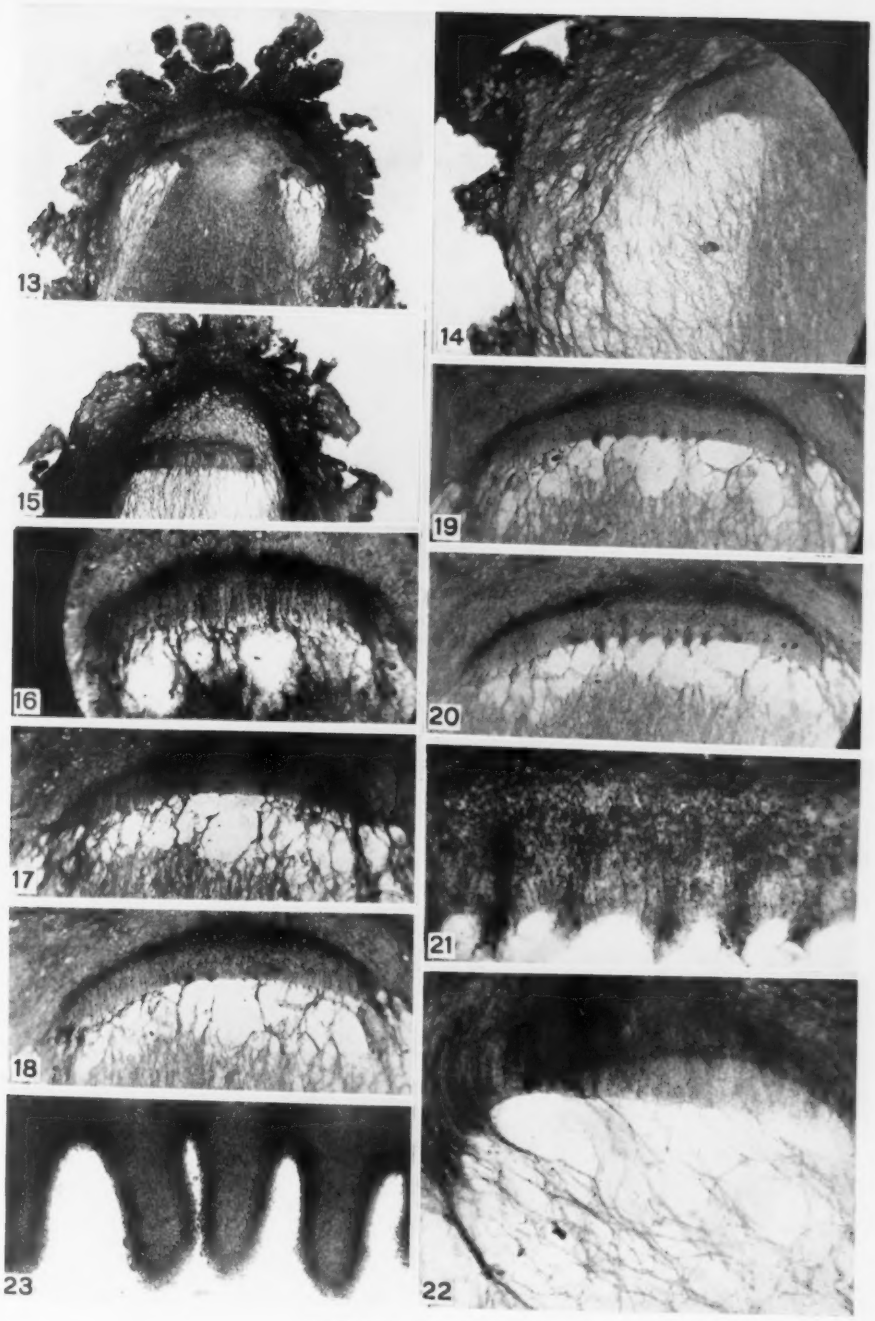
FIG. 47.—Left side of median section; note that hymenophore is decurrent on stem at this stage; gill cavity is clearly differentiated and free from ground tissue; ×154.

FIG. 48.—Tangential section near pileus margin; uneven hyphae of hymenophore primordium are aggregated into tufts; gill cavity is just beginning to form by tearing away of ground tissue from hymenophore; ×154.

FIG. 49.—Section of same fruit body, but nearer stem; palisade forming in middle region of hymenophore surface; ×93.



SAWYER on PHOLIOTA



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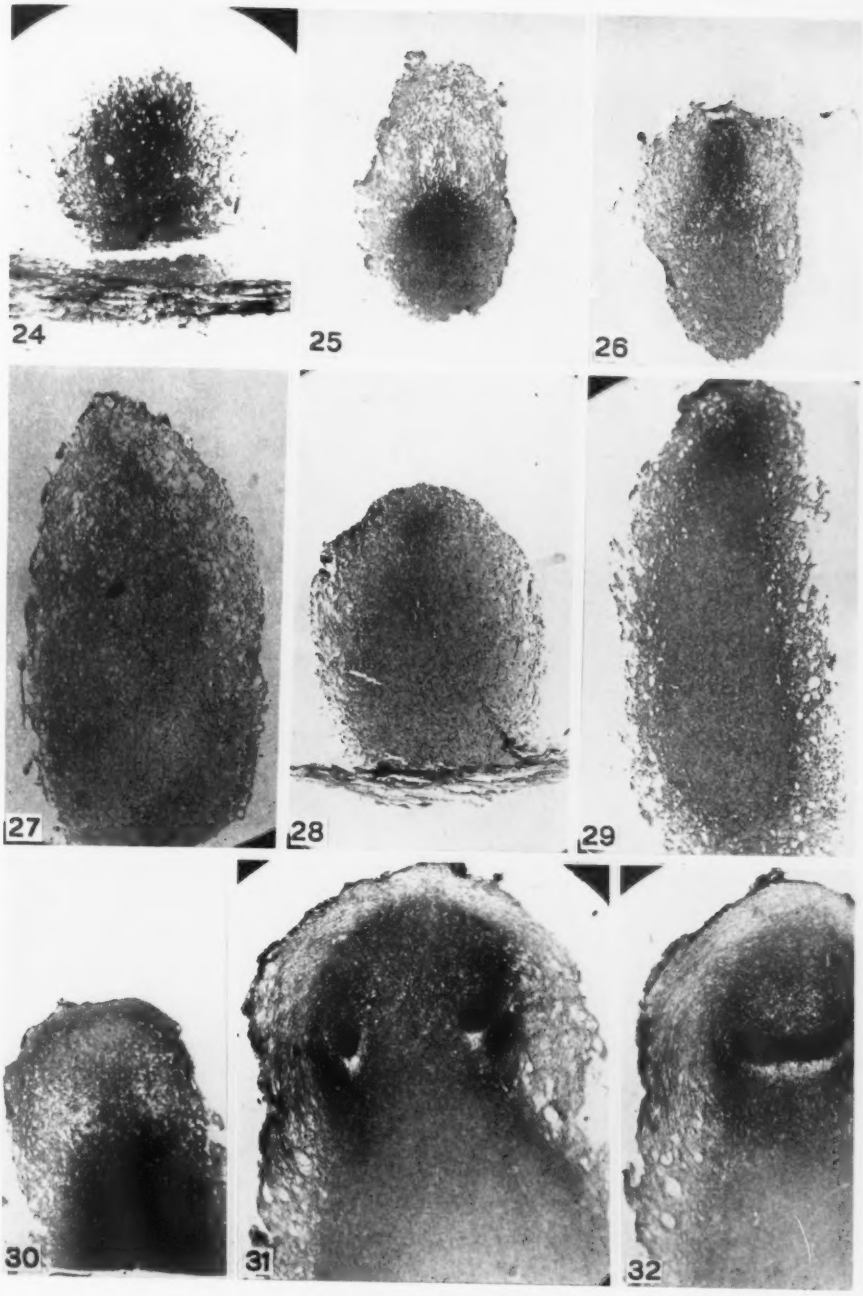

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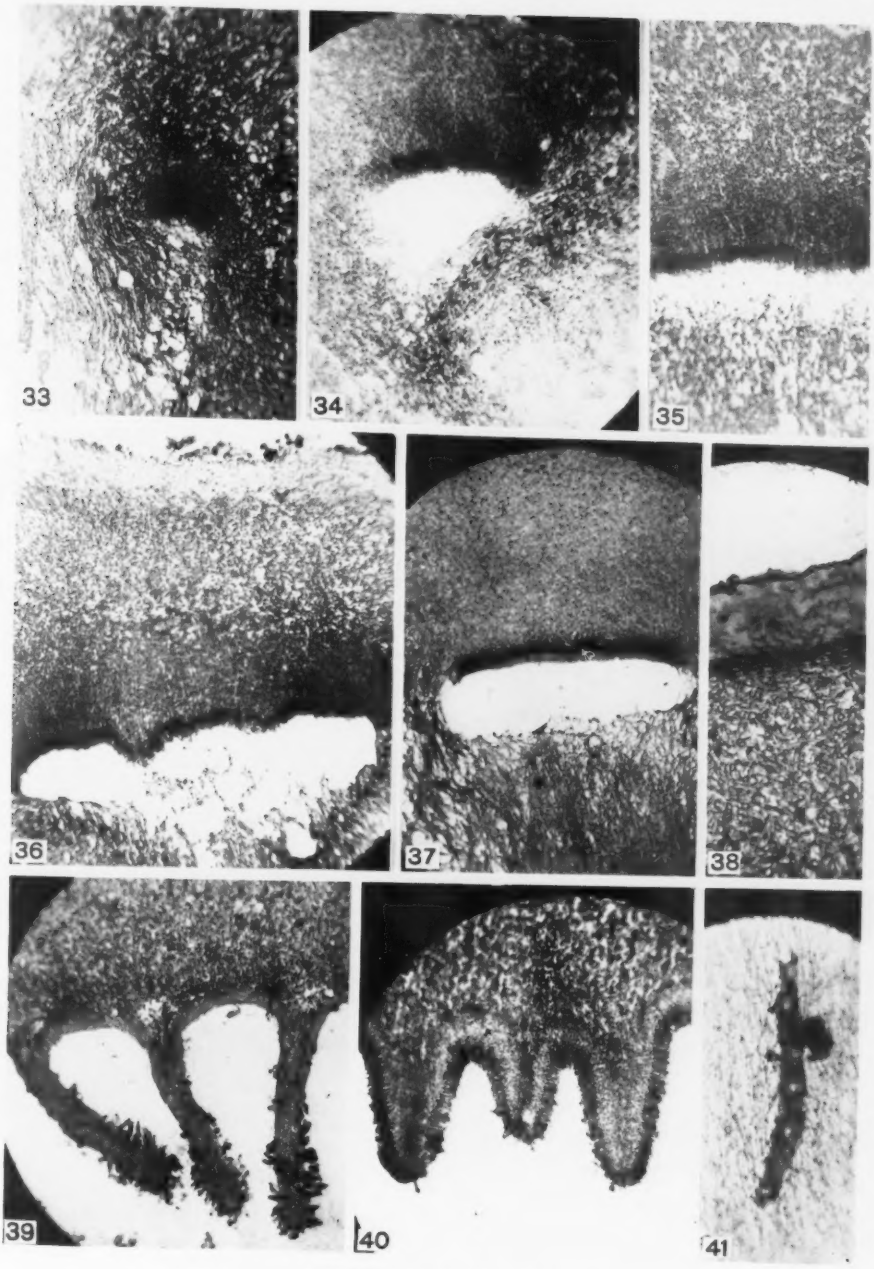

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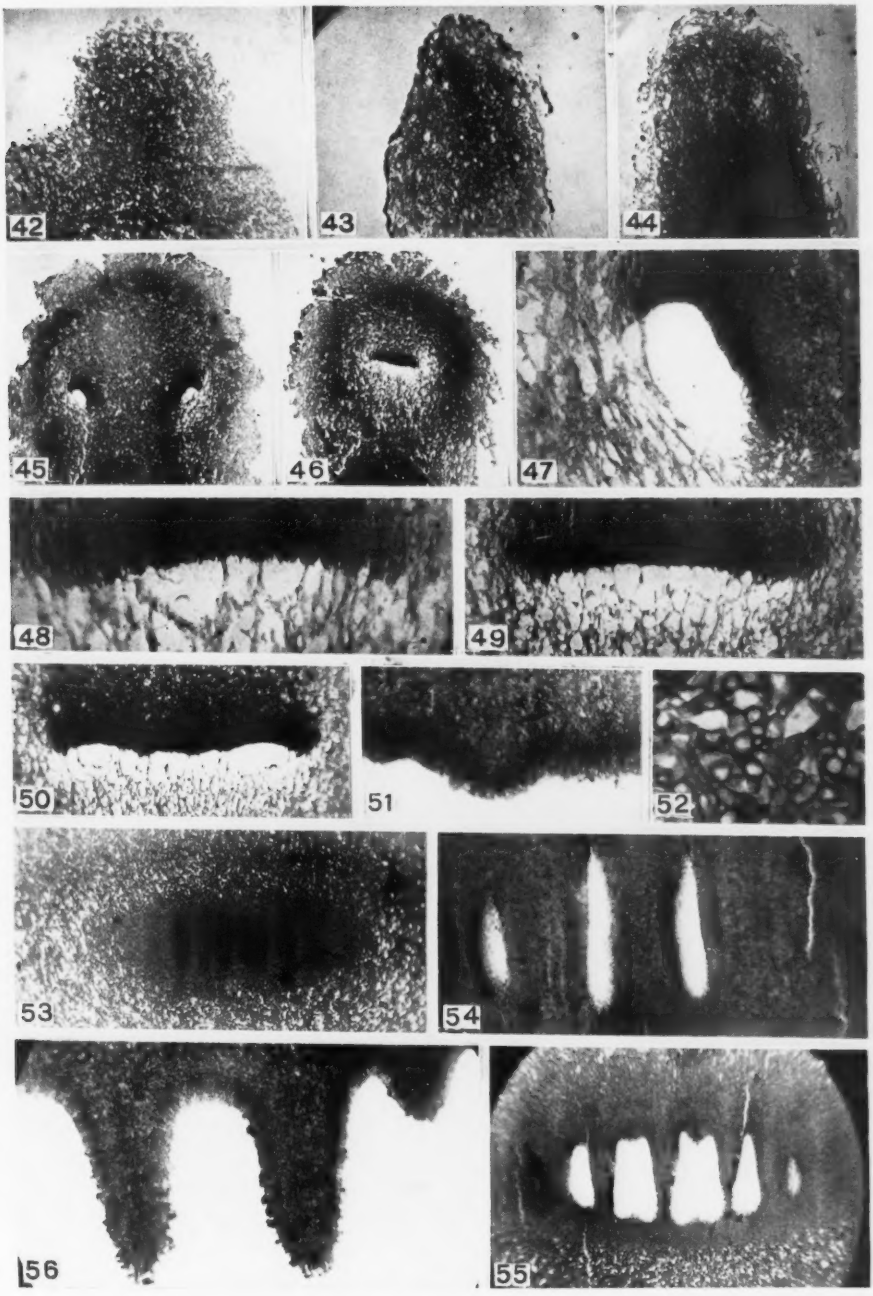

SAWYER on PHOLIOTA

FIG. 50.—Same, nearer stem; note the 3 slight downward projections of palisade, which are very young gill salients; some ground tissue still clings to them because their downward growth relieves tension upon it to some extent; ×67.

FIG. 51.—Section from same fruit body near stem, showing very broad gill salients; ×250

FIG. 52.—Large, thick-walled cells of blematogen; ×250.

FIGS. 53–55.—Sections tangential in enrolled pileus margin; fig. 53 (×33) has not cut into gill cavity; "backs" of gills show as deeper stained areas; in fig. 54 (×154) gill cavity is reached and gill is shown in middle with trama in center and palisade on either side; its origin is in hymenophore both above and below; fig. 55 (×56) is nearer stem.

FIG. 56.—Tangential section, showing well-formed gill, with trama and palisade; ×154.

METHODS OF STUDYING PERMEABILITY OF PROTOPLASM TO SALTS

S. C. Brooks

Investigation of the permeability of protoplasm to electrolytes has led to many apparent conflicts between evidence secured by different methods and between the theoretical conclusions based thereon. An intensive study of the evidence, and of the methods themselves, has shown that these apparent conflicts are in large measure due to an imperfect understanding of the limitations of the methods or to unwarranted assumptions as to the nature and reactions of living matter. It is therefore of interest to consider critically the methods heretofore employed in the study of permeability in order to determine which of these methods may be considered most reliable, and thus to acquire a broader understanding of the problem, and to lay the foundation for further investigation.

The methods employed in the investigation of the permeability of protoplasm to electrolytes fall into 4 general categories, in which the criteria employed are: (1) chemical analysis of tissue extracts or of solutions bathing the tissues, (2) visible changes within the cell, (3) turgidity of cells or tissues, and (4) electrical conductivity of tissues or of masses of cells. To these may be added a diffusion method, which will be described by the writer in a subsequent paper.

Chemical analysis

Analysis of tissues or tissue extracts.—Perhaps the earliest employment of a method for the detection of an inorganic salt within a living cell which had previously been bathed by a solution of that salt was that by Janse (**16**). Filaments of a species of *Spirogyra* were caused to burst in distilled water containing diphenylamine. Cells which had been bathed in a potassium nitrate solution for some hours previous to testing gave a strong positive reaction in the extruded cell material at the moment of bursting, while those briefly immersed in the potassium nitrate solution, and

then tested, gave no reaction. This method, although positive, can yield only qualitative data.

As a method for the investigation of protoplasmic permeability, quantitative analysis of tissue extracts involves several important sources of error, among which may be mentioned the presence of salts in the intercellular spaces and in the cell walls, where they may be held in solution or by adsorption, variations of the concentration and constitution of expressed juices dependent on the pressure used in extraction (cf. MAMELI **30**), and, most serious of all, adsorption or chemical union of the salts within the cell. Thus, while aluminium ions might displace potassium ions in an adsorption compound, sodium ions might displace the potassium ions to a small extent only. In this way the free aluminium content of the cell would remain low, and the original rate of endosmosis of aluminium salts would be maintained, while that of sodium salts would steadily decrease as the free sodium content of the cell increased. A similar effect might be produced by the formation of hydrates of aluminium and sodium; the former being insoluble would form a precipitate, while the latter would remain in solution.[1] The relative permeability of the tissues to different salts would then be made to appear other than it actually was. The last error also affects methods involving the amount of salt taken by tissues from a solution. Conclusions as to the permeability of the plasma membrane, therefore, cannot be safely based upon data furnished by experiments of this type, such as those of NATHANSOHN (**38**, pp. 453 ff.), PANTANELLI (**51**), DE RUFZ DE LAVISON (**55, 56**), COLIN and DE RUFZ DE LAVISON (**4, 5**), MEURER (**36**), and many others.

PAINE (**50**), using these methods, drew the conclusion that yeast cells are wholly impermeable to inorganic salts.[2] He found a slight absorption of these salts by the yeast cells, but attributed it to adsorption of the salts in the cell walls. Irrespective of the validity of his interpretation, there is no evidence that it is applicable to plants in general.

[1] Precipitates have been observed to form in living cells when they are treated with aluminium salts, but the nature of these precipitates has not been ascertained.

[2] Aqueous solutions of NaCl, $(NH_4)_2SO_4$, Na_3AsO_4, Na_2HPO_4, and sodium hexose phosphate were used.

ANALYSIS OF SOLUTIONS BATHING THE TISSUES.—The method of analysis may also be applied to the diffusion from living cells ("exosmosis") of substances normally present in the cells and retained by the impermeability of the protoplasm (they may accumulate in the cell walls of terrestrial plants in quantities sufficient to maintain a condition of equilibrium with the solution inside the cell, and may diffuse out when the cells are placed in water). Under certain conditions these substances may be made to diffuse from the cells in appreciable quantities. The experiments of WÄCHTER (**69**) on the exosmosis of sugar from onion bulb scales seemed to indicate that this exosmosis was inhibited by various salts. In the light of more recent evidence it seems possible that this was due to antagonization of traces of toxic salts in the "Leitungs-Wasser" which he used.

Other experiments have dealt with the absorption of salts from the solution as well as with exosmosis. The results of the experiments of TRUE (**63**), TRUE and BARTLETT (**64, 65, 66**), and MERRILL (**34, 35**), like those of WÄCHTER, were visible only after several hours, and the intervening effects upon permeability could not be determined. There was also opportunity for "regulative processes" and other complications to influence the absorption of salts to a marked extent during this interval, and a probability that some of the external cells would be killed and would give off their contained solutes to the surrounding solution. It is quite probable that these effects are of importance in experiments of such long duration as those of the investigators mentioned. The most serious objection to using the analysis of the solution as a criterion of permeability is that the method does not distinguish between permeability and absorption. These two things have little to do with each other. If the absorbed substance is trapped within the cell (by precipitation, or by a chemical change preventing it from diffusing out), it will continue to diffuse in, while a substance which is not trapped will soon stop diffusing in. Hence we see that absorption is no criterion of permeability, although it is so used by many investigators. The absorption of a substance may be great when permeability is small, and vice versa. The same objection applies to some extent to the use of exosmosis as a criterion, since

increased exosmosis may be due, not to increased permeability, but to increased production within the cell of the substance which diffuses out.

Visible changes within the cell

This method, although sometimes valuable in the investigation of the penetration of substances like the alkaloids which form intra-vitam precipitates, and acids and alkalies which cause color changes of pigments or intra-vitam stains, has found little application in the study of the penetration of inorganic salts.

OSTERHOUT (**39**) showed that crystals of calcium oxalate form in the root hairs of seedlings of *Dianthus barbatus* (previously grown in distilled water) within a few hours after their immersion in dilute solutions of calcium salts, and the subsequent normal growth of the cells proved that they were not injured. ENDLER (**7**) followed microscopically the entrance of intra-vitam stains (neutral red and methylene blue) into various plant cells under the influence of various kations. He also investigated the rate of disappearance of the dyes from stained cells, living and dead. The experiments are extremely instructive, showing that at 24 or more hours the passage of dyes through the membrane was increased by kations in the following order: $Na < K < Mg < Ca < Al$. Aluminium formed an exception in that at very low concentrations the exit of dyes from the living cells was inhibited. This inhibition was not observed in the experiments with dead cells, where the influence of the kations was due only to their physical action on the colloidal dye tannate formed in the cells. This series is precisely what would be expected of experiments of long duration on the supposition that a temporary decrease in permeability was produced by all the polyvalent kations, and that this was followed by an increase. Extreme dilution would prolong the period of decreased permeability, and would account for the inhibition of exosmosis which ENDLER found to occur when extremely dilute solutions of aluminium salts were used.

The interesting experiments of LOEB (**29**) on the diffusion of neutral red through the egg membrane of a marine teleost fish (*Fundulus* sp.), a process which occurs readily in electrolyte solutions but very slowly in distilled water, are apparently concerned

with the permeability of a membrane considerably different from the plasma membrane. LOEB suggests the theory that the dye kation is held in the membrane in a combination with a colloidal anion, and that this combination is broken down by the anions of a surrounding salt solution. The behavior of the potassium ion is shown to be like that of the dye kation, at least in its initial stages. Similar processes may occur in the plasma membrane, but it is not possible to apply LOEB's conclusions directly to the behavior of unspecialized protoplasm.

HARVEY (**12**) has studied the permeability of plant cells to alkalies by introducing an intra-vitam stain, neutral red, which turns yellow in the presence of alkalies.[3] It was found that ammonia and the amines penetrated living and dead tissues with equal and very great rapidity, while the strong bases, although penetrating dead cells with great rapidity, required much longer to penetrate living cells. It seems possible that penetration of bases at the concentration used (0.025 N) was due to injury of the cells.

Turgidity of cells or tissues

The typical living cell behaves toward osmotically active solutions as though it were surrounded by an elastic semipermeable membrane. In view of the widespread confusion regarding the osmotic relations of living cells, it seems necessary to analyze, in so far as the present imperfect state of our knowledge of the physical laws governing osmotic phenomena will allow, the behavior of a cell which acts as a simple osmometer. It will then be possible to judge more accurately the value of the data furnished by the many methods based upon the study of the osmotic relations of living cells. Such a typical cell may be pictured as a body of solution surrounded by an elastic membrane permeable to and bathed by the solvent (in this case water), and slightly if at all permeable to the contained solute. Water will enter such a cell until the internal hydrostatic pressure produced by the tension of the stretched membrane just overcomes the tendency of water to enter the cell. There will thus arise a condition of equilibrium which will be main-

[3] Neutral red changes color between H ion concentrations of 6×10^{-6} and 1×10^{-8}N.

tained unless there is either a change in the tendency of the water to enter, or a change in the tension of the membrane. The latter has not been shown to occur, but may be responsible for certain as yet unexplained phenomena observed in plasmolytic experiments. The former will be produced by alterations in the concentration of the solution bathing the cell, an increase in its concentration causing a loss of water from the cell, with consequent shrinkage, and a decrease causing an intake of water with accompanying increase in volume. These changes will proceed until a new equilibrium is established at which the internal osmotic pressure is again equal to that of the external solution plus the pressure produced by the tension of the membrane.

The rate at which the exchange of water will occur is a function of the difference in osmotic pressure between the intra- and extra-cellular solutions and of the permeability of the membrane to water. There appears to be a tendency among physiologists to confuse the effect of the rate of penetration of a solute on that of water (which is produced by the resultant progressive change in the osmotic gradient), with a hypothetical effect, independent of the osmotic gradient, produced by the simultaneous passage of both solute and solvent through the membrane. There is no physical justification for the latter assumption, and the two ideas should be carefully distinguished. The change in volume of the cell is the sum of the change in volume due to diffusion of water and that due to diffusion of the solute. In cases where the solute is a substance like alcohol, the latter factor may be of considerable importance; but protoplasm is in general so much less permeable to inorganic salts than to water that their diffusion may be neglected in so far as their volume is concerned.

The intra- and extra-cellular osmotic pressures are thus quickly equalized by passage of water through the membrane, and a state of equilibrium is reached, which, if the membrane is permeable to water only, is permanent. But if a diffusion of solute occurs, it will cause changes in osmotic pressure which will lead to further water exchange, and this process will proceed until the solute attains an equal concentration in both intra- and extra-cellular solutions, and a true equilibrium is thus established. The rate at

which these changes occur will depend upon the permeability of the protoplasm to the solute and upon the concentration gradient causing the diffusion of the solute.

If a living cell be placed in a fairly concentrated salt solution, the salt usually diffuses into the cell (a process known as "endosmosis"), while the sugars, to which a large part of the intracellular osmotic pressure was originally due, remain for the most part within the cell. Under these conditions the cell will increase in volume, until it reaches the same turgidity (that is, the same degree of distension due to the tendency of water to enter the cell) which it would have possessed had there been no salt at all present. The outward diffusion of salts or other substances from the cell ("exosmosis") is usually negligible, but it is always to be remembered that such a diffusion may be occurring simultaneously with the endosmosis. If a salt after entering the cell forms there osmotically inactive compounds, either by adsorption or by chemical combination, and does not at the same time cause the liberation of an osmotically equivalent amount of some other substance, the turgidity of the cell is less than would be expected, and there is a decrease in the apparent rate of penetration of the salt.

If a plant cell be placed in a solution which causes shrinkage, the cell wall will contract elastically for a certain distance, and will then suffer no further change in size; meanwhile the continued shrinkage of the protoplasm will cause it to retract from the cell wall. This separation of the protoplasm from the cell wall is known as plasmolysis. It was first observed by PRINGSHEIM (**52**) in 1854, and was ascribed by NÄGELI (**37**) to the impermeability of the protoplast to the plasmolyzing substance. In this process the protoplasm may tear away just inside the cell wall, leaving attached to it a thin layer of protoplasm to which the central mass remains for a time connected by fine threads (cf. BOWER **1**, CHODAT and BOUBIER **3**, HECHT **13**, and KÜSTER **22**). The process of plasmolysis may then subject the protoplasm to a very considerable mechanical injury, and it is quite probable that its subsequent permeability will not be the same as that of a protoplast which has not been subjected to plasmolysis. In animal cells and tissues, where no cell walls are present, and in tissues of plants when shrinkage is not

carried far enough to cause plasmolysis, we have a means of avoiding this objection. We may consider first those methods in which plasmolysis occurs.

METHODS INVOLVING PLASMOLYSIS

1. *Concentration Required to Produce Plasmolysis.*—DE VRIES (**67**) noticed that the concentration of a glycerine solution just concentrated enough to produce plasmolysis was higher than that expected from the calculated osmotic pressure of the solution. He attributed this to the penetration of glycerine into the cell.

On the assumption that an increase in the concentration of a given substance required to produce plasmolysis indicates an increase of permeability, LEPESCHKIN (**23, 24, 25, 26**) and TRÖNDLE (**62**) claim to have demonstrated an increase of the permeability of the protoplasm due to increased illumination; and ECKERSON (**6**) seeks the cause of the thermotropic curvatures of roots in an increase in permeability due to rise in temperature. By the same method KREHAN (**20, 21**) has studied the effect of potassium cyanide on the permeability of cells of *Tradescantia discolor*, the experiments seeming to indicate that dilute solutions (0.001 M) of potassium cyanide cause a temporary and reversible increase in permeability, and that this is followed by a decrease in permeability which begins simultaneously with loss of the reversibility.

OSTERHOUT (**40**) has shown that solutions of sodium and calcium chlorides, either of which alone is unable to produce plasmolysis (of cells of *Spirogyra* sp.), may cause rapid plasmolysis when mixed in such proportions that the ratio of sodium atoms to those of calcium is about 20 to 1. Since the normal permeability of the protoplasm is most nearly attained in the mixed solution, which is a partially balanced mixture, it would appear that the permeability of the protoplasm was abnormally high in one of the pure solutions. It is possible, however, to establish a different interpretation of this phenomenon; this will be considered in the light of evidence secured by other methods.

FLURI (**9**) found that aluminium salts so altered the protoplasm of certain plant cells as to make it impossible to plasmolyze them.

This alteration he supposes to be the production of complete permeability. SZÜCS (**61**) has since stated that the alteration consists of a hardening of the protoplasm, since centrifuging no longer displaces the cell contents. He also found the "hardening" to be temporary, and to be followed by "reliquefaction."

LEPESCHKIN (**27**) claims to determine with great accuracy, by a method based upon the difference in the osmotic pressures of isotonic plasmolyzing substances, the absolute rate of penetration of these substances. It is impossible to explain the method clearly and at the same time briefly, but its essential features are as follows: a comparison of the osmotic pressure of a saccharose solution which will just cause visible plasmolysis, with that of a glycerine solution which, following the saccharose, will cause no change in volume (as determined by LEPESCHKIN'S criterion) shows the latter to be the higher. If we let μ represent a factor proportional to the permeability of the protoplasm to the glycerine, and assume that the protoplasm is impermeable to saccharose, then $\mu=\frac{C^1-C}{C^1}$, where C^1 is the concentration of glycerine found to be isotonic with the saccharose solution, and C the concentration calculated to be isosmotic with the saccharose solution. For saccharose we may substitute any substance to which the protoplasm is supposed to be impermeable, and for glycerine any substance whose rate of penetration it is desired to measure.

This method would be exact provided the following assumptions were in accord with the facts: (1) the protoplasm is impermeable to the control substance (in this case saccharose); (2) neither of the substances used causes any alteration in the permeability of the protoplasm; (3) no exosmosis occurs. All these assumptions are rendered highly improbable by the evidence already secured by other methods, and additional evidence against their validity will be submitted by the writer in a subsequent paper.

LEPESCHKIN also appears to assume that there is an effect on the water equilibrium caused by the simultaneous diffusion of solvent and solute through a membrane, and independent of the progressive changes in the osmotic gradient thus arising. This assumption is, as has been previously pointed out, without physical

basis. The method of LEPESCHKIN is therefore of extremely doubtful value.

2. *Recovery from Plasmolysis.*—Recovery of plasmolyzed cells was first noted by KLEBS (**17**) in 1887, who found that glycerine was able to penetrate the plant cell. He was unable to detect recovery of cells plasmolyzed by solutions of potassium nitrate or sodium chloride. DE VRIES (**68**) obtained similar results at about the same time. JANSE (**16**), whose work has been quite generally overlooked, demonstrated the penetration of potassium nitrate, sodium chloride, and saccharose by observations on the recovery of plasmolyzed cells of the marine algae *Chaetomorpha aerea* and *Dictyota* sp., and *Spirogyra nitida*, *Tradescantia discolor*, and *Curcuma* sp. It was thus conclusively shown that at least some inorganic salts can penetrate living cells of many types of plants.

OVERTON (**48**) was unable to observe any cases of recovery of cells plasmolyzed by inorganic salts. He supposed this to be due to the insolubility of such salts in lipoid substances, which he supposed to constitute the plasma membrane. It has been pointed out by OSTERHOUT (**41**) that OVERTON in all probability overlooked the recovery of the cells which he used, confusing the subsequent "false plasmolysis," due to the injury of the cells, for a continuation of the true plasmolysis. OSTERHOUT showed that a great variety of salts penetrate and cause recovery. OSTERHOUT also showed that the rate of recovery of *Spirogyra* cells was more rapid when a salt of one of certain monovalent kations was used to produce plasmolysis than when a calcium salt was similarly used.[4] It was impossible to establish more than the most general quantitative relations in these experiments. Recently FITTING (**8**) has conducted an extensive series of investigations on the permeability of cells of *Tradescantia discolor* L'Heritier (*Rhoeo discolor* Hance). His data may be most easily understood if stated graphically. Comparable strips of epidermis were plasmolyzed in a series of solutions differing by equal increases in molecular concentration. If there was no difference in the rate of recovery, a curve in which the ordinates represented recovery time and the

[4] The solutions used in these experiments were in each case of the lowest concentration which would produce plasmolysis.

abscissae concentration of the plasmolyzing solution would be a straight line. It was found, however, that such a curve was concave to the axis of the ordinates. This indicated a decrease in the rate of recovery with time. FITTING considers this to establish the fact that such salts cause a progressive decrease in the permeability of the protoplasm. He considers the possibility that exosmosis might have occurred in his experiments, and cites experiments which supposedly show that all possible exosmosis had taken place during the preliminary 4–6 hours' exposure of the tissues to distilled water. There are serious discrepancies in his data, such as the fact that a solution of a higher osmotic pressure is required to produce plasmolysis in tissues from which all possible exosmosis is supposed to have taken place than is required to produce it in otherwise comparable tissues from which no exosmosis has occurred. It is probable that FITTING has some important variables in the method which he has employed, and since he has failed to investigate the effect of salts of monovalent kations on exosmosis, it is probable that the supposed decrease of endosmosis is in reality an increase of exosmosis, which would have the same effect on the rate of recovery. FITTING also states that the cells are wholly impermeable to salts of bivalent and trivalent kations, with the possible exception of strontium. This is in conflict with the experiments of OSTERHOUT.

METHODS NOT INVOLVING PLASMOLYSIS

In rapidly elongating plant tissues there is usually a very considerable pressure exerted by the protoplasts against the cell walls which confine them. If all the cell walls of the stem are thin and elastic, the whole stem will be kept in a stretched condition by this pressure. The presence of thick-walled cells, such as fibrovascular or epidermal cells, which do not yield to internal pressure, will, if they are symmetrically distributed, prevent this elongation of the tissue. If we cut such a stem or peduncle so that these two types of tissue are unsymmetrically distributed, the whole tissue will curl so that the elastic tissue forms the longer or convex side. The distention of the elastic tissues, and therefore the degree of curvature, will vary with the turgidity of the tissues. A hypertonic

solution will withdraw water from the cells, and consequently reduce the turgidity and the degree of curvature, while a hypotonic solution will have the opposite effect. The penetration of the protoplasm by a salt with whose solution such a tissue had come into osmotic equilibrium would lead to an increase in the turgidity, and hence in the curvature of the tissue. DE VRIES (**67**), in the investigation of the isotonic coefficients of various substances by this method, observed such a secondary increase in curvature. Such tissue curvatures have not since been used in quantitative researches on the permeability of the protoplasm. The writer, however, has found it possible to make use of this method for quantitative determinations of permeability (BROOKS **1a**).

Changes in the volume or weight of animal cells or tissues have been used by many investigators to determine the rate of penetration of electrolytes. Red blood corpuscles and striated muscle have been the most frequently used materials.[5] As an example of the former, the work of KOZAWA (**19**) may be quoted. This investigator added to 1 cc. of corpuscles centrifuged from defibrinated blood of various mammals 2 cc. of various solutions of equal osmotic pressure (as judged by the freezing point depression). The corpuscles were again centrifuged after a time varying from 15 minutes to 23 hours, and the volume of the mass of corpuscles noted.[6] Increase of volume was considered to indicate penetration of the solute. Sodium salts were not observed to cause any increase in volume. KOEPPE (**18**) made similar observations.

In some animals glucose appeared to penetrate; in others it did not. It was found to be impossible to influence the permeability to glucose by various agents, including certain inorganic salts. These conclusions agree with those obtained by the use of quantitative analytical methods, notably those of GYÖRGY (**10**), who was unable to influence the rate of penetration of glucose into red blood corpuscles by suspension of the corpuscles in buffer solutions of

[5] Changes in weight of whole organisms have been used in the study of osmotic relations. Cf. OVERTON (**48**), QUINTON (**53**), and HENRI and LALOU (**15**).

[6] The determination of volume changes in red blood corpuscles by centrifuging was first suggested by HEDIN (**14**), and is known as the "haematocrit" method. Similar methods have been applied to other free cells such as leucocytes and spermatozoa. Cf. HAMBURGER (**11**).

various hydrogen ion concentrations, or in solutions containing Ca, Mg, Mn, oxalate, or SO_4 ions (cf. also MASING **31** and LOEB **28**).

OVERTON (**49**) made successive determinations of the weight of sartorius muscles of the frog during treatment with various solutions. He reports that no increase in weight took place in a 0.7 per cent sodium chloride solution during a period of many hours; that isotonic solutions of the phosphate, tartrate, sulphate, ethyl sulphate, and acetate of potassium induced no change in weight during 50 hours. After a few hours an increase of weight occurred in solutions of potassium chloride, iodide, bromide, and nitrate, but OVERTON found these changes to be irreversible, and concludes that the normal muscle is impermeable to neutral salts. SIEBECK (**57**), on the other hand, finds that under proper conditions the increase in weight of kidney tissue in a pure isotonic solution of potassium chloride is reversible, and therefore considers that these cells are normally permeable to potassium chloride. In general the permeability of animal cells to neutral salts seems to be less, and more often characterized by selective peculiarities than that of plant cells. The red blood corpuscles, for example, may well be considered to be surrounded by a considerably specialized protoplasmic envelope.

The experiments of LOEB (**29**) on the permeability of fertilized *Fundulus* eggs to electrolytes are concerned with a peculiarly specialized envelope surrounding the embryo. This envelope is characterized by an exceedingly small permeability to salts. Thus an embryo 4–14 days old within the egg membrane survives 3 days of exposure to a solution (50 cc. 3 M NaCl+1 cc. 10/8 M $CaCl_2$) which is almost instantly fatal to the newly hatched fish. As has previously been noted, generalizations as to the permeability of protoplasm cannot be made from data furnished by experiments on such a membrane, and a more extended discussion of the results of these experiments would not be profitable here.

Electrical conductivity of tissues or of masses of cells

The conduction of electrical current by a solution involves the passage through the solution of electrically charged atoms of

some substance. These charged atoms, known as ions, are not created by the electrical conditions imposed, but already exist in all solutions capable of conducting a current. The rate at which the current will be conducted by the ions of a given salt will depend upon two factors, the potential gradient and the frictional or other resistance to the migration of the ions. If the potential gradient be kept constant, we may follow fluctuations in the last factor by a measurement of the current, or by a direct measurement of the electrical resistance. If, therefore, we force the current to pass through living protoplasm in a solution, the resistance offered by the protoplasm to the passage of the ions will measure its permeability to the ions in question (the permeability may be regarded as varying inversely as the resistance). By the use of alternating currents of rather high frequency we avoid large effects due to accumulation of ions at surfaces impermeable to them.[7]

A method of this type was independently employed at about the same time by RÓTH (**54**), BUGARSKY and TANGL (**2**), and STEWART (**58**), who found that the conductivity of blood serum was greater than that of blood itself, and that the resistance rose rapidly with increase of the proportion of corpuscles to serum. The blood corpuscles seemed to be slightly or not at all permeable to the electrolytes of blood. The conductivity of the suspension of corpuscles was shown to be increased by haemolytic agents, the corpuscles then being permeable to salts (cf. WOELFEL **70**, also STEWART **58, 59**). MCCLENDON has also attempted to study the changes in permeability of sea urchin eggs during fertilization (**32**) and of muscles in tetanus (**33**). The evidence from his experiments on sea urchin eggs agrees with that of HARVEY (**12**), previously mentioned, but difficulties in technique which MCCLENDON found it impossible to avoid make the data of these experiments exceedingly unreliable.

The experiments of OSTERHOUT (**42–47**) on the conductivity of tissue of the marine alga *Laminaria* have shown the important fact that the permeability of living protoplasm is altered by salts in pure and mixed solutions in a manner characteristic of the ionic constitu-

[7] The small capacity effect will be proportional to the resistance, so that no relative error is thereby introduced.

tion of the solution. The kations are of particular importance. All monovalent kations (excepting the hydrogen ion) produce only an increase in permeability of the protoplasm. This increase, reversible in its first stages, finally leads to death and complete permeability. Bivalent and trivalent kations and the hydrogen ion cause a temporary and reversible decrease of permeability which is followed or superseded by an increase which is irreversible and leads to death of the cells. In a balanced solution such as sea water the resistance remains constant provided the laboratory conditions are such as to maintain the full vitality of the tissue. We have here a method of determining quantitatively the permeability of the protoplasm at any instant, and the data secured demonstrate the extreme importance of progressive changes in the permeability of protoplasm. It would be possible to imagine that the passage of an electrical current through the tissues was responsible, at least in some measure, for the observed changes in permeability. It would be of advantage, therefore, to check the results of OSTERHOUT'S method by the use of some method entirely free from this possible objection. The method is also applicable to certain types of tissue only, and it is desirable to extend to other types of plants the principles derived by the application of this method.

The plasmolytic experiments of OSTERHOUT (40) may be explained in the light of the experiments by the conductivity method in the following manner. During the time required to produce plasmolysis the permeability has considerably increased in the sodium chloride solution and somewhat decreased in the calcium chloride solution. In that time much more sodium chloride has penetrated the cell, therefore, than of the salts of the mixed solution in which the permeability remains normal, and these again more than the calcium chloride, and the osmotic gradients have changed accordingly. The osmotic pressures of the solutions which will produce visible plasmolysis will then have suffered an increase over the actually isosmotic solutions, and in this order: calcium chloride very little, the mixed solution slightly more, and sodium chloride considerably more. If we now mix a large amount of sodium chloride of a concentration just insufficient to produce plasmolysis with a small amount of a similar calcium chloride

solution, the resulting osmotic pressure will be considerably above that of a similar mixed solution (that is, one just insufficient to cause plasmolysis), and plasmolysis will result.

Summary

From a consideration of the methods heretofore used in the study of permeability it would appear that the steps most essential to further progress toward the solution of the problem are: (1) a thorough analysis of the various disturbing factors in the methods involving chemical determinations and the satisfactory interpretation of the results secured by such methods; (2) the same type of analysis of the methods depending on turgor, with special reference to the possible effect of exosmosis; and (3) the establishment of methods of determining progressive changes in permeability without the various disadvantages of the other methods.

The writer hopes to show in subsequent papers that the diffusion method, which he has devised, answers these requirements, and that it is also possible to interpret satisfactorily the data obtained by certain methods dependent upon the use of turgor as a criterion.

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BRIEFER ARTICLES

NOTES ON EFFECT OF DYES ON *ENDOTHIA PARASITICA*

Some experiments were made in growing the chestnut blight fungus, *Endothia parasitica*, in a liquid medium to which stains had been added. The dyes were congo red, trypan blue, methylene blue, and neutral red plus 7 per cent NaCl, all "vital stains." They were added to a nutrient medium, Pasteur's solution. This solution was not a particularly good one for the cultivation of the fungus. Congo red, trypan blue, and methylene blue were used in 1/1000 of 1 per cent solutions. The dilution of neutral red plus 7 per cent NaCl was not known. This solution had been successfully used in the vital staining of some animals of the lower orders, and it was tried on the fungus by diluting it 1 cc. to 500 cc. of Pasteur's solution. The cultures were started from conidia mixed with a little mycelium taken from a test tube culture.

Record of experiments

CONGO RED (1/1000 of 1 per cent congo red[1] in Pasteur's solution).—Conidia germinated and produced normal mycelium. The hyphae became red colored. When the cultures were 5 days old, hyphae and medium had the same color. Reaction of medium acid to litmus paper. Eleven days after inoculation the medium had turned a pale yellow color, almost colorless, and clear. The medium was acid to litmus paper. The red colored mycelium which had been spreading steadily over the surface of the medium showed in sharp contrast. Seven days later the fully and normally developed fungus had produced pycnidia and conidia. The mycelium in the cultures appeared a pinkish red, and the pycnidia looked yellow. Under the microscope, the color of the hyphae was red to opaque. The colored hyphae turned blue at once on being placed in $\frac{1}{3}$ per cent sulphuric acid and later lost their color. The pycnidia on being tested in sulphuric acid varied in reaction; some of them turned blue, some did not. The conidia in the crushed fruits showed no color.

TRYPAN BLUE (1/1000 of 1 per cent trypan[2] blue in Pasteur's solution).—All the conidia stained a deep blue, so that their growth could

[1] G. Grübler and Company.

[2] Meister Lucius and Brüning Hoechst.

be followed readily. They swelled, germinated, and produced normal mycelia. The hyphae were a deep blue; the growing tips were opaque to pale blue. Their blue color deepened with age. About 12 days after inoculation the medium lost its blue color, turning light yellow; the blue hyphae with uncolored pycnidia floated on its surface.

METHYLENE BLUE (1/1000 of 1 per cent methylene blue[3] in Pasteur's solution).—The majority of conidia did not germinate, but instead developed vacuoles. The hyphae produced did not stain, except when the cells were dead. The plasmolyzed contents of the dead cells gathered in a blue clump in the center. These isolated dead cells distributed throughout the mycelium gave it a pale blue color. While the cultures grew more vigorously than the controls, they did not grow as well as the congo red and trypan blue cultures. No fruits were produced.

NEUTRAL RED (neutral red plus 7 per cent NaCl solution diluted 1 to 500 parts Pasteur's solution).—A few conidia germinated and grew. The cultures were as good as the controls. No fruits. Hyphae uncolored.

CONTROL (Pasteur's solution).—Conidia germinated, produced mycelium, but no pycnidia.

In the case of the congo red and trypan blue cultures, it was thought that the mycelium had gradually stored up all the dye in the medium. Neutral red and salt did not stain. In those cases in which methylene blue penetrated the cells, it apparently was fatal. The fact that the solutions containing the stains supported the fungus better than the control medium seems to indicate one of two things: either the toxicity of the dyes in the concentrations used (with the exception of the neutral red and salt) was enough to be a stimulus, or the dyes may have counteracted the elements in Pasteur's solution inhibitive to the growth of the fungus and so allowed the mycelium a better development.

PFEFFER,[4] in a series of experiments with methylene blue, found the dye accumulated in the cell sap rather than in the protoplast of the cell. The substances which render the storage possible, he says, are not always identical; the two which are best known are tannin and phloroglucin. Mycelium taken from cornmeal agar cultures was tested for the presence of tannin and phloroglucin. There was no evidence of these two substances in the vegetative cells. The reagents used were ferric sulphate, hydrogen peroxide and ferrous sulphate, copper acetate, ferrous sulphate, and boiling potassium bichromate.

[3] G. Grübler and Company.

[4] PFEFFER, W., Untersuchungen Botanischen Institut zu Tübingen. 1886. II.

Mycelia growing in a congo red 1 to 200 parts maltose solution were examined to see whether the dye had accumulated in the vacuoles or protoplast of the cell. The mycelium had not behaved with methylene blue as with the two colloids, but this gave no indication as to the deposition of the pigments.

The fungus grew in the solution, but not vigorously. The mycelium was so deeply stained as to be reddish black. Under the microscope the conidia and older cells of the hyphae were dark red, while the youngest cells were a pale pink. Treatment with 50 per cent nitric acid showed by its blue colored reaction that most of the pigment was in the walls of all the cells, only less in the younger cells. The hyphae were plasmolyzed with a NaCl solution and also by drying; the contracted protoplasm in the center of the cells was red, the cell wall looking white in contrast. This first was noticed in the younger cells, the quantity of pigment in the older cell walls having obscured the color of the protoplast, until the last stages of plasmolysis had been reached. Sulphuric acid, nitric, or hydrochloric produced besides a blue color, what was thought might be a blue precipitate. These very small spots, seen with the oil immersion lens, were on the cell walls and inside on the plasmolyzed protoplast. Glycerin caused the color to stream from the mycelium. Sodium hydroxide, while it brightened the red, also caused the color to diffuse into the surrounding solution. Throughout all these reactions glistening white granules in the protoplasts could be seen.

It would seem from these reactions that a great deal of the congo red accumulated in the cell walls, some passed inside the cell walls, where it appeared as though the protoplast had stored the dye in the form of minute granules. In the nutrient solutions containing congo red, the difference in the ability of fungi to store stain was so marked that contaminations could be seen at once. For instance, *Penicillium* sp., yeast, and a rod-shaped bacterium found growing in them remained unstained until dead.

It is suggested that *Endothia parasitica* (Mur.) A. and A. may be a good subject for the study of mitochondria in fungous cells.—CAROLINE RUMBOLD, *Botanical Laboratory, University of Pennsylvania.*

CURRENT LITERATURE

BOOK REVIEWS

The study of plants

WOODHEAD[1] has published an elementary textbook of botany which seems to possess several features deserving better things than this tardy notice. It is a pleasure to note that emphasis is placed upon the work of plants and their relation to the habitat. Form is considered in relation to function, and the amount of microscopic work is reduced to a minimum. The book is divided into 5 parts, the first being devoted to the structure and function of the vegetative organs, occupying 150 pages. About half as much space is devoted to the reproductive organs, and here, as before, attention is confined to seed plants. The next 100 pages contain a characterization of the great groups of systematic botany, including a special consideration of trees and shrubs. The final portion is an introduction to the study of plant communities, including those of the roadside and cultivated fields. One of the commendable features of the book is the choice of so much material from the common plants of the field and wayside. It is a relief to see such a large number of new illustrations, including some good photomicrographs replacing the stock figures that reappear so persistently in most textbooks.

The final chapters are well organized to introduce the concepts and principles of plant associations, and possess the further advantage that the examples are chosen from the vegetation of the British Isles, thus affording an opportunity of following principles involved rather than copying directly the lessons outlined in the text. This among other things will certainly make the book indispensable to the teacher in search of new ideas and fresh exercises with which to enliven his present program.

It would not be difficult to find points to be criticized, but they are not on the whole serious defects. As an example, there is the use of "abnormal forms" to describe roots that are not of the most common fibrous type. The difference in the terminology from that commonly used in this country would probably be a serious difficulty in introducing it as a text for class use, but as a book of secondary reference and as a source of different ideas and new material it should not be overlooked.—GEO. D. FULLER.

[1] WOODHEAD, T. W., The study of plants; an introduction to botany and plant ecology. 8vo. pp. 424. *figs. 258*. Oxford: Clarendon Press. 1915. $1.40.

The life of inland waters

This book[2] attempts to present in an elementary manner a comprehensive view of our fresh waters as the abode of a teeming multitude of organisms. The viewpoint is essentially ecological, with a background of economic possibilities. The first portion of the volume consists of an introduction to the subject, that is to the science of limnology, including a historical sketch of important epochs in its development and a glance at the present facilities for study, which include some 30 biological field stations in the United States and Canada. There follows a discussion of water with respect to its transparency, temperature, and circulation, as well as its gas, mineral, and organic content. The various types of aquatic habitats are then presented. These include ponds, lakes, streams, marshes, and bogs.

In the consideration of aquatic organisms, 57 pages are devoted to plant and 83 to animal forms. Concerning the latter the reviewer will venture no opinion, but the paragraphs on plant life seem interesting and accurate, but so general that they would need to be supplemented by good teaching in the field to accomplish their purpose. In such an ecological textbook the adjustment of organisms to the varied conditions of aquatic life under which they exist, and the interaction of the aquatic communities are, of course, the most important parts of the book, and considering the immensity of the field to be covered and the complexity of the material it would seem that the authors have been very successful. Still the book will have to be supplemented by a teacher with an unusually wide acquaintance with both plant and animal life in order to be the success which the subject deserves.

The final chapter upon the possibilities of water crops and water culture reveals economic possibilities of no small importance. The entire volume is in non-technical language, the material is well organized, and the contents may be appreciated by a person with a minimum of scientific attainment, while at the same time few teachers will find themselves sufficiently equipped to teach the entire field effectively. Good illustrations and a fairly extensive bibliography add to the value of the volume.—GEO. D. FULLER.

MINOR NOTICES

Fruit diseases.—A recent textbook by HESLER and WHETZEL[3] discusses the subject from an essentially New England viewpoint, omitting from all consideration fruits and diseases that are of major interest in many states and nearly all that are of interest in our tropical possessions. The discussion of such diseases as are treated is in many instances from a local viewpoint rather

[2] NEEDAM, JAMES G., and LLOYD, J. T., The life of inland waters. 8vo. pp. 438. *figs.* 244. New York: Comstock Co. 1916. $3.00.

[3] HESLER, LEX R., and WHETZEL, HERBERT RICE, Manual of fruit diseases. 12mo. pp. xx+462. *figs.* 126. New York: Macmillan. 1917.

than of a general nature. This is especially obvious in the treatment of such diseases as apple rust and pear blight. The illustrations are poor. Such figures as 29, 32, 40, 69, 76, 83, 86, 92, 98 are not worthy of publication. Throughout the book there is a tendency to present various conflicting theories and arguments concerning a given disease, with the result that often the real conclusions, if any, are buried or obscured. This really is the result of the status of pathology, of insufficient knowledge of the diseases in question, but the value of such presentation to the student and especially to the practical grower is doubtful. Assuming a central New York viewpoint and interest, the book may be said to give a very complete presentation of what is known of fruit diseases, with valuable lists of references to original sources of information. It is, as the authors announce, the first American text to deal wholly with diseases of fruits, and here for the first time are brought together with comprehensive discussion many obscure and little-known diseases. The facts presented are well selected, and the book constitutes a valuable addition to the literature of plant diseases. —F. L. STEVENS.

North American Flora.—The second part of Vol. 21 contains the presentation of Amaranthaceae by STANDLEY,[4] who recognizes 166 species distributed among 21 genera. *Amaranthus* is much the largest genus, with 42 species, followed by *Iresine* with 32, *Achyranthes* with 31, and *Gomphrena* with 15. These 4 genera contain 120 of the 166 species, the remaining 46 being distributed among 17 genera. New species, 10 in number, are described in *Amaranthus* (4), *Acnida*, *Froelichia*, *Achyranthes*, *Gomphrena* (2), and *Iresine*.—J. M. C.

North American Flora.—The second part of Vol. 10 contains the presentation of Agaricales by MURRILL,[5] including the subtribe Pluteanae. The largest genera are *Entoloma* (63 spp.), *Pluteus* (57 spp.), and *Leptoniella* (43 spp.). Ten genera are presented, and 109 new species are distributed as follows: *Claudopus*, *Eccilia* (9), *Leptoniella* (14), *Nolanea* (11), *Pleuropus* (7), *Entoloma* (34), *Pluteus* (30), *Chamaeota*, and *Volvariopsis* (2).—J. M. C.

NOTES FOR STUDENTS

Anthocyans.—Since the review of the anthocyan (anthocyanin) pigments by CROCKER,[6] much of interest from the chemical point of view has appeared. As pointed out by CROCKER, these facts are of marked significance to all botanists. Students interested in the general problems of anthocyans (botanical, chemical, and genetic) will find much of value in Miss WHELDALE'S book.[7]

[4] STANDLEY, PAUL C., North American Flora **21**:part 2. pp. 95–169. (Chenopodiales) Amaranthaceae. New York Botanic Garden. 1917.

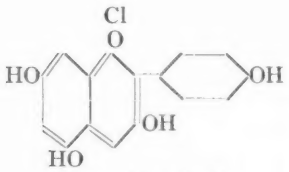
[5] MURRILL, W. A., North American Flora **10**:part 2. pp. 77–144. Agaricales: Agaricaceae (pars); Agariceae (pars). New York Botanic Garden. 1917.

[6] CROCKER, WM., BOT. GAZ. **61**:349. 1916.

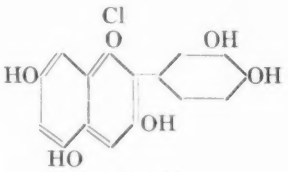
[7] WHELDALE, M., The anthocyanin pigments of plants. Cambridge University Press. 1916.

The bibliography is very complete and should stimulate further work. Brief reviews of the anthocyan pigments have recently appeared by ATKINS,[8] EVEREST,[9] WHELDALE,[10] TRUTTWIN,[11] and WEST.[12]

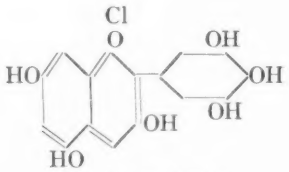
The constitution of the anthocyanidins (the sugar-free compound, obtained upon hydrolysis of the anthocyan with 20 per cent hydrochloric acid) has been definitely established by the synthesis of cyanidin (by the reduction of quercetin with magnesium at 35°),[13] and of pelargonidin (in which phloroglucinic aldehyde was the starting point).[14] These syntheses prove the correctness of the formulas earlier proposed by WILLSTÄTTER, after studying the physical and chemical properties of the anthocyanidin chlorides. The mechanism of the reduction of quercetin and other flavones to anthocyanidins has been questioned by certain Japanese workers,[15] who claim to have isolated various magnesium compounds as intermediate products. These criticisms, however, in no way invalidate the general conclusion as to the structure of the anthocyanidins.



Pelargonidin chloride



Cyanidin chloride



Delphinidin chloride

[8] ATKINS, W. R. G., Researches in plant physiology. Whittaker Co. 1916.

[9] EVEREST, A. E., Science Progress **9**:597. 1914–15; Jour. Genetics **4**:367. 1914–15.

[10] WHELDALE, M., Jour. Genetics **4**:109, 369. 1914–15.

[11] TRUTTWIN, HANS, Chem. Zeit. **40**:478. 1916.

[12] WEST, CLARENCE J., Register of Phi Lambda Upsilon **6**:1–18. 1917.

[13] WILLSTÄTTER, R., and MALLISON, H., Sitz. Ber. Akad. Wiss. **12**:769. 1914.

[14] WILLSTÄTTER, R., and ZECHMEISTER, L., Sitz. Ber. Akad. Wiss. **12**:886. 1914.

[15] SHIBATA, K., SHIBATA, Y., and KASIWAGI, I., Jour. Pharm. Soc. Japan no. 417. 1916; Chem. Abstr. **11**:578. 1917.

The amyl alcohol test for anthocyans has played an important part in the course of WILLSTÄTTER'S work. The anthocyan (glucoside) remains quantitatively in dilute (2 N) sulphuric acid when shaken with amyl alcohol, whereas the anthocyanidin (non-glucoside) passes quantitatively into the alcoholic layer, yielding a red solution. When shaken with a solution of sodium acetate the red color becomes violet or violet red, the pigment remaining quantitatively in the amyl alcohol. On shaking with sodium carbonate, the alcohol solution turns blue or bluish-green, and at the same time the pigment descends quantitatively into the aqueous layer.

This is true only for diglucosides. Monoglucosides yield a certain amount of the pigment to the amyl alcohol. Rhamnose glucosides (containing one molecule each of rhamnose and glucose) behave like the monoglucosides. From this it is seen that the test may be used to distinguish nomo- and rhamnose-glucosides from diglucosides.

The test is also of service in testing the individuality of anthocyan. For this the acid used must be of sufficient concentration to prevent the conversion of the colored chloride into the colorless carbinol (ψ base) and yet must be dilute enough to dissolve the chloride readily (0.5 per cent hydrochloric acid is recommended). The solutions must be very dilute on account of the limited solubility of the coloring matter in amyl alcohol. The intensity of the color of the amyl alcohol solution is compared with that of the anthocyan chloride. Two successive extractions with amyl alcohol are made, and the fraction of the anthocyan in the extracts, called the "partition number," should be the same in both cases.

WILLSTÄTTER'S first work indicated that the flower and fruit pigments might fall into 3 classes, depending upon whether the sugar-free component was pelargonidin, cyanidin, or delphinidin. The possibility of the other classes was not excluded, but they were not found. The second series of papers would seem to confirm this.[16] Here, also, only pigments belonging to one of these three classes were found. It is true that the possibility of other classes is, again, not excluded. Indeed, WILLSTÄTTER states in the article on the poppy that the preliminary work on different varieties of this flower has indicated that the isolation of several new anthocyanidins may be expected. The one flower studied, however, was found to contain cyanidin.

Table I gives the pigments thus far isolated and studied, together with the formula of chloride, the source from which it was isolated, the anthocyanidin resulting on hydrolysis and the sugar component. They are divided into the 3 classes mentioned.

[16] WILLSTÄTTER, R., Untersuchungen über die Anthocyane. XI (with BOLTON, E. K.). Ann. **412**:113. 1916; XII (with BOLTON, E. K.). *Ibid.* 136; XIII (with BURDICK, C. L.). *Ibid.* 149; XIV (with ZOLLINGER, E. H.). *Ibid.* 164; XV (with WEIL, F. J.). *Ibid.* 178; XVI (with ZOLLINGER, E. H.). *Ibid.* 195; XVII (with BURDICK, C. L.). *Ibid.* 217; XVIII (with WEIL, F. J.). *Ibid.* 231.

The relationships of these various anthocyans and anthocyanidins may best be seen from the diagrams on page 259.

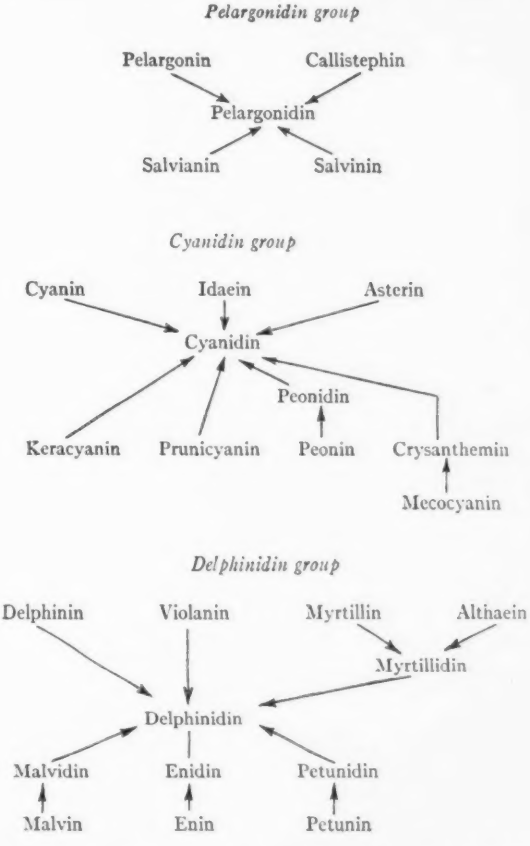
TABLE I

Anthocyan	Formula	Anthocyanidin	Occurrence	Sugar components
Pelargonidingroup				
Pelargonin	$C_{27}H_{31}O_{15}Cl$	Pelargonidin	Scarlet red pelargonium, cornflower, orange red and dark violet dahlia	2 glucose
Callistephin	$C_{21}H_{21}O_{10}Cl$	"	Summer aster	1 "
Salvianin	.....	"	Scarlet red salvia	2 "
Salvinin	$C_{27}H_{31}O_{15}Cl$	"	Salvia	2 "
Salvin	$C_{27}H_{27}O_{13}Cl$	"	Salvia	
Cyanidin group				
Cyanin	$C_{27}H_{31}O_{16}Cl$	Cyanidin	Cornflower, rose, dark red garden dahlia	2 glucose
Idaein	$C_{21}H_{21}O_{11}Cl$	"	Whortleberry	1 galactose
Chrysanthemin	$C_{21}H_{21}O_{11}Cl$	"	Chrysanthemum	1 glucose
Asterin	$C_{21}H_{21}O_{11}Cl$	"	Summer aster	1 "
Keracyanin	$C_{27}H_{31}O_{15}Cl$	"	Cherry	Glucose and rhamnose
Prunicyanin	$C_{27}H_{31}O_{15}Cl$	"	Black thornberry	Rhamnose and a hexose
Mecocyanin	$C_{27}H_{31}O_{16}Cl$	"	Poppy	2 glucose
Peonin	$C_{28}H_{33}O_{16}Cl$	Peonidin*	Peony	1 "
Delphinidin group				
Delphinin	$C_{31}H_{39}O_{21}Cl$	Delphinidin	Larkspur	2 "
Violanin	.....	"	Pansy	Rhamnose and glucose
Enin	$C_{23}H_{23}O_{12}Cl$	Enidin*	Grapes	1 glucose
Malvin	$C_{29}H_{35}O_{17}Cl$	Malvidin*	Wild mallow	2 "
Myrtillin	$C_{22}H_{23}O_{12}Cl$	Myrtillidin*	Bilberry	1 "
Althaein	$C_{22}H_{23}O_{12}Cl$	" *	Hollyhock	1 "
Petunin	$C_{28}H_{33}O_{17}Cl$	Petunidin*	Petunia	2 "
Ampelopsin	$C_{22}H_{23}O_{12}Cl$	Ampelopsidin*	*Ampelopsis* (wild grapes)	1 "

* Mono—or dimethyl ethers.

A study of table I shows the remarkable distribution of each group. Thus, cyanidin glucosides have been isolated from the following sources: the blue corn flower contains 0.7 per cent of the dry weight of the flower, while the Bordeaux red variety contains 13–14 per cent; 2 per cent of the dry weight of the rose (commercial *Rosa gallica*) and 20 per cent of the dark red garden dahlia are cyanin. Cyanidin has also been isolated from the chrysanthemum, the summer aster, the poppy, and the peony. Varying amounts of cyanidin glucosides are found in the following: *Zinnia elegans* Jacq., *Gaillardia bicolor* Hook., *Helenium autumnale* L., *Gadiolus* (the scarlet varieties of which contain a pelargonidin glucoside), *Tulipa Gesneriana* L., *Tropaeolum majus* L., *Ribes*

rubrum L., the raspberry, and the berry of the mountain ash. These glucosides also occur very extensively in fruits and in yellowish red, red, brown, and dark blue berries. They have been isolated from the cranberry, the cherry, the sloe (black thornberry), and the plum. The occurrence of a pure red coloring



matter in the bluish black skins of such fruits is remarkable. Some explanation has to be found as to how cyanidin is able to impart such deep colors to fruits. The other groups show almost as wide a distribution as does the cyanidin group.

The conclusion that the color of the flower depends, in part, upon the reaction of the cell sap seems to need some modification, in view of the work of

HAAS[17] on this question. Determination of the total acidity and actual acidity by means of titration, and the gas chain and buffer solutions, shows that the reaction of the cells studied ranges from about P_H+3 to P_H+7. If we call the buffer solution acid up to P_H+7, neutral when at P_H+7, and alkaline when higher than P_H+7, it is evident that it is unsafe to call cell sap acid when red, neutral or alkaline when blue, and markedly alkaline when green, unless the color changes of the particular pigment are first studied by some method, such as that of using buffer solutions of known hydrogen ion concentration.

One other study may also be mentioned in this connection. These chemical investigations inspired a study by SHIBATA, NAGAI, and KISHIDA[18] of the physiological and biological significance of the anthocyans and flavones in plants. The evidence obtained established a somewhat unexpected fact, namely, that the flavone derivatives are one of the cell contents of very common occurrence in the plant kingdom. In fact, they are quite as common as chlorophyll, tannins, sugars, starches, and proteins. They are not only found in the yellow coloring matter, but also in the cell sap of the epidermis and in the underlying tissue of plants in general. In only a small number of plants is a complete absence of chromogen proved; for example, in the white corn flower (*Centaurea cyanus*), oxalis (*Oxalis violacea*), pink (*Dianthus caryphyllus*), and pelargonium (*Pelargonium cucullatum*). The function of the flavone derivatives dissolved in the cell sap is to protect the living protoplasm and the important biochemical agents involved from the injurious action of the ultraviolet rays of sunlight by absorbing them at the peripheral layer of the plant organism. The plausibility of this assumption is justified by an extensive study of the flora of alpine and tropical regions. The green leaves of deciduous trees, which produce anthocyan pigments in autumn, contain a considerable amount of flavones. The production of autumnal color is due to the biochemical change, that is, the reduction of already existing flavones in the leaf, initiated by the physiological condition at the end of the growing season, without having special ecological significance.

This brief survey of recent progress in anthocyan chemistry makes it seem very probable that in the near future we may have a complete classification of these pigments, based upon accurate and definite knowledge of the chemical structure of the substances involved. It is to be hoped that the work may make substantial progress in spite of the war.—CLARENCE J. WEST.

Taxonomic notes.—EVANS[19] has published a monograph of the North American species of *Marchantia*, recognizing 9 species, one of which (*M. breviloba* of Jamaica) is new. The extensive discussion of species is preceded

[17] HAAS, A. R., Jour. Biol. Chem. **27**:233. 1916; BOT. GAZ. **63**:232. 1917.

[18] SHIBATA, K., NAGAI, I., and KISHIDA, M., Jour. Biol. Chem. **28**:93. 1916–17.

[19] EVANS, ALEXANDER W., The American species of *Marchantia*. Trans. Conn. Acad. Sci. **21**:201–313. *figs. 20*. 1917.

by an account of the morphology of the genus. The detailed description of each species is accompanied by the synonymy, citations of exsiccatae, and a very full discussion.

In a revision of *Herberta*, the same author[20] discusses 4 species, 2 of which are described as new.

FERNALD[21] has described a new *Cardamine* (*C. Longii*) from Maine, which grows in "shaded rock-pockets and crevices covered at high tide." In the description it is contrasted with *C. pennsylvanica*.

MACKENZIE[22] has described a new species of *Carex* (*C. convoluta*), which ranges from Maine to Manitoba and south to the Gulf States. Heretofore it has been included in *C. rosea*.

PENNELL,[23] in continuation of his studies of the plants of the southern states, has described a new *Smilax* (*S. leptanthera*) from Georgia, closely allied to *S. tamnifolia*.

RENDLE[24] has described a new genus (*Maidenia*) of Hydrocharidaceae from West Australia, belonging to the Vallisnerieae.

ROCK,[25] in connection with the preparation of a monograph of certain genera of the Lobeliaceae of Hawaii, has published 9 new species and varieties of *Cyanea*, and 2 new varieties of *Lobelia*.

WIEGAND[26] has described a new species of *Eragrostis* (*E. peregrina*), which occurs as a weed in this country, as well as in Germany and Japan, and which is unknown as yet in an indigenous state. It was separated from the well known *E. pilosa* of Europe and America, and had been named *E. pilosa* var. *condensata* Hackel.—J. M. C.

A peculiar spore distribution.—According to ROMELL,[27] the brown powder commonly observed covering the upper surfaces of sporophores of

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[20] EVANS, ALEXANDER W., Notes on the genus *Herberta*, with a revision of the species known from Europe, Canada, and the United States. Bull. Torr. Bot. Club **44**:191–222. *pl. 8*. 1917.

[21] FERNALD, M. L., A new *Cardamine* from southern Maine. Rhodora **19**:91, 92. 1917.

[22] MACKENZIE, K. K. Notes on *Carex*. X. Bull. Torr. Bot. Club **43**:428. 1916.

[23] PENNELL, F. W., Notes on plants of the southern United States. II. Bull. Torr. Bot. Club **43**:412. 1916.

[24] RENDLE, A. B., *Maidenia*, a new genus of Hydrocharidaceae. Jour. Botany **54**:313–316. *pl. 545*. 1916.

[25] ROCK, JOSEPH F., Notes on Hawaiian Lobelioideae, with descriptions of new species and varieties. Bull. Torr. Bot. Club **44**:229–239. *pls. 9–16*. 1917.

[26] WIEGAND, K. M., A new species of *Eragrostis*. Rhodora **19**:93–96. 1917.

[27] ROMELL, LARS, Hvarifrån kammer det Bruna Pulvrit å öfre Sidan af *Polyporus applanatus* och andra *Ganoderma*-arter? Svensk. Bot. Tidskr. **10**:340–348. 1916 (with English summary).

some species of *Fomes* consists of true basidiospores which have been carried upward by gentle currents of air, such as arise from differences of temperature at different levels, and have lodged on the pilei. In support of this view the author points out that other objects in the vicinity of the fungi also become covered with spores. A simple experiment lends further support to this view. Pieces of cardboard pinned on the surface of sporophores of *Polyporus applanatus* in May were covered with spore powder in July, as were also all portions of the surfaces of the fruit bodies except the areas covered by the paper. While not venturing to explain the pertinaceous adherence of the spores to surfaces, the author suggests that they stick fast by virtue of a gelatinous cutin layer. Regarding the spore powder on the surface of these fungi, mycologists have generally adopted the view of SCHULZER, according to which the powder consists of conidia whose origin on the surface of the pileus is minutely described by him. Although opposing the view of SCHULZER, ROMELL does not speak of repeating the histological examination of that investigator. If the explanation of ROMELL is correct, it is a matter for inquiry why this peculiarity of spore distribution is restricted to a few members of the genus *Fomes* and does not occur more generally among the Hymenomycetes. Even among the caespitose Agaricaceae, only those parts of the pilei overtopped by others are usually covered by spores, while the exposed parts generally remain free.—H. HASSELBRING.

Journal of Forestry.—With the issue of January 1917 the *Journal of Forestry*[28] takes the place of the *Proceedings of the Society of American Foresters* and of the *Forestry Quarterly*. This change is in no sense an absorption of either journal by the other, but rather an amalgamation of the best features of the two original publications is intended. In continuing the activities of the two parent publications it is expected that the new one will contain 800 to 1200 pages per annual volume.

The first number of the new journal sets a high standard of excellence which promises well for its future. Among its leading articles is a critical review of 30 years of forestry work of the Federal Government by FERNOW. Some slight idea of the progress involved may be formed from the increase in appropriations from $10,000 in 1886 to over $5,000,000 at present. In addition to the Federal organization, 30 states have organized forest departments and 13 now possess state forests. There is also a thorough discussion of "continuous forest production of privately owned timberlands" by KIRKLAND, and an excellent critical review of recent papers on the moisture withholding power of soils by MOORE. Perhaps still more useful to botanists and ecologists is an extended glossary of the technical terms of forestry now used by the profession in America.

[28] Journal of Forestry. 8 monthly issues per year. Pub. by the Society of American Foresters. Washington, D.C. $3.00 per year.

It is safe to predict that the new journal will be of increasing interest to all botanists, and more particularly so to ecologists who see in forestry the practical application of their more theoretical studies.—GEO. D. FULLER.

Endemism and the mutation theory.—WILLIS, in papers previously reviewed in this journal,[29] working upon the flora of Ceylon, has proposed the theory that relative endemism is determined by relative age, the youngest species being the endemics. RIDLEY[30] points out that WILLIS has based his arguments upon statistics gathered from herbarium specimens; and illustrates that such will not agree with field statistics, the commonest species sometimes being poorly represented in the herbarium. In connection with his theory WILLIS states that "very common" plants could not disappear without a geological catastrophe. This RIDLEY has shown to be inaccurate, illustrating from his own personal experience and from well known historical evidence that common species have disappeared within a few years, due to parasites, the activities of man, and relatively slight climatic changes. RIDLEY claims that the Ceylon endemics are relics, since there are no other local species from which they could have been evolved recently.

The remainder of the paper is a criticism of the mutation theory as used by WILLIS to explain the origin of the Ceylon flora. RIDLEY's arguments and evidence are of the characteristic Neo-Darwinian type. As an attack upon the mutation theory, or WILLIS' application of it, the paper is unconvincing to the reviewer.—MERLE C. COULTER.

Heath and grassland.—FARROW[31] has described an interesting area of Norfolk and Suffolk Counties, England, where upon sandy soil with only 22.5 inches of annual rainfall there develops a transition from a heath dominated by *Calluna vulgaris* to a grassland with a short close turf in which *Festuca ovina* and *Agrostis vulgaris* are the most abundant species. The sterility of the soil is such that some has never been cultivated and much of the rest once farmed has long since been abandoned. The grassland seems to present the nearest approach to continental steppe conditions to be found in Great Britain.

In the second of his papers the author finds that the chief factor in the invasion of the heath by the grassland is the destruction of the *Calluna vulgaris* by an overpopulation of rabbits. Once the *Calluna* becomes weakened by its leaves being eaten by the rabbits, a luxuriant growth of *Cladonia* appears to be able to smother it and to hasten its death. In the absence of rabbits

[29] Rev. in BOT. GAZ. **61**:82. 1916; **62**:160. 1916; **63**:419. 1917.

[30] RIDLEY, H. H., On endemism and the mutation theory. Ann. Botany **30**:551–574. 1916.

[31] FARROW, E. P., On the ecology of the vegetation of Breckland. I. General description of Breckland and its vegetation. Jour. Ecology **3**:211–228. 1915; II. Factors relating to the relative distribution of *Calluna* heath and grass heath in Breckland. Jour. Ecology **4**:57–64. 1916.

the *Calluna* maintains its dominance over both the grasses and the lichens.—GEO. D. FULLER.

The variable desert.—Writing in semipopular language, HARRIS[32] has described the wide variation of climatic and other factors influencing plant life in the desert region of Tucson, Arizona, in such a way as to give a more graphic and living picture of this most interesting region than will be found in other more voluminous and technical reports. The wide variation of precipitation from year to year and from month to month is made clear by a diagram, while the large proportion of waste of the scanty water supply is emphasized. The wide range of temperature during both the year and the day, the almost infinite variety of plant forms, extending from thin to thick-leaved herbs, from broad-leaved to leafless shrubs, and from succulent to woody plants with varied aspect at different seasons of the year, are all clearly depicted. In a word, the reader is made to appreciate some of the complexity of environment and diversity of organisms which have rendered this region so fascinating to the intelligent layman and to the investigating scientist.—GEO. D. FULLER.

Ecology of lichens.—In connection with a systematic study of the lichen flora of South Lancashire, WHELDON and TRAVIS[33] discuss some of the factors detrimental to the growth of these plants. Particular attention is directed to their sensitiveness to pollution of the atmosphere by the smoke and chemical fumes attendant upon the development of a manufacturing industry. The observations are of a general rather than of a particular character and are not accompanied by any experimental data. They also note that a calcareous substratum seems to counteract the effect of smoke upon the lichens. The selective action of these plants is well illustrated by the group of species peculiar to the carboniferous limestone.—GEO. D. FULLER.

Tolerance of trees.—After making studies of light in the forests of Michigan and Vermont and its effect upon the growth, BURNS[34] concludes that "tolerance" used to express a light relationship should no longer be used in reference to the development of tree seedlings. He found the filtered light in the forest of little value in the decomposition of carbon dioxide compared with the weakened white light. What is usually regarded as a light relationship is really the total relationship of a tree to all factors of the habitat. He further concludes that light readings in the forest are of little value.—GEO. D. FULLER.

[32] HARRIS, J. ARTHUR, The variable desert. Scientific Monthly **3**:41–49. 1916.

[33] WHELDON, J. A., and TRAVIS, W. G., The lichens of South Lancashire. Jour. Linn. Soc. **43**:87–136. 1915.

[34] BURNS, G. P., Studies in tolerance of New England forest trees. III. Discontinuous light in forests. Vt. Agric. Exp. Sta. Bull. 193. pp. 23. 1916.

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